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ARKIV FÖR KEMI Band 3 nr 1

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# Derivate von Reduktonen und Dienen

Von H. v. Euler, Hans Hasselquist und Armin Glaser

Mit 2 Figuren im Text

## A. Triose-Redukton, p-Aminobenzoesäure und p-Aminosulfosäure

Wie in einer vorhergehenden Arbeit bereits erwähnt wurde, sind hinsichtlich des aus Triose-Redukton und p-Aminobenzoesäure (p-ABS) entstehenden Produktes verschiedene Resultate erhalten worden: Während Euler u. Hasselquist[1] in Übereinstimmung mit Angier et al.[2] (Am. Cyanamide Comp.) sowie mit Bell, Cocker und O'Meara[3] ein aus 1 Mol. Reduktion und 1 Mol. p-ABS bestehendes Produkt $C_{10}H_9O_4N$ gewannen, und zwar auch, wenn in der Reaktionsmischung p-ABS im Überschuss vorhanden war, beschrieben Forrest und Walker[4] ein Kondensationsprodukt (Schmpt. 240–250°) vom Komponenten-Verhältnis 2 p-ABS: 1 Redukton (N gef. 8,3 %; ber. für $C_{17}H_{14}O_5N_2$ N 8,6 %).

Wir erhielten die Verbindung Redukton-p-ABS als mikroskopische, orange-gelbe Nadeln vom Schmpt. 264° (unter Zersetzung), schwerlöslich in Wasser und in den meisten organischen Lösungsmitteln ausser Pyridin, reichlich löslich in konc. Schwefelsäure mit orangegelber Farbe; mit Wasser ausfällbar. Die gesättigte wässrige Lösung reagiert sauer, etwa pH 6,5 entsprechend. Die Alkalisalze sind leicht löslich, aber schon bei sehr schwachem Ansäuern (pH = 6,5) fällt Redukton-p-ABS flockig aus. Eine Fluorescenz wurde nicht festgestellt.

Redukton-p-ABS ist in mehrfacher Hinsicht von wesentlichem biochemischen Interesse, zunächst als Ausgangsmaterial für die Darstellung von Pteridinen[2], sodann besonders durch die Ergebnisse von O'Meara et al. über die Rolle der Redukton-p-ABS als Wuchsstoff bei der Entwicklung von Bakterien[3], z. B. von Streptococcen (Lancet, 1947 II. 747).

Die bakteriostatische Wirkung der Sulfonamide wird seit Woods[5] darauf zurückgeführt, dass die den Wuchsstoff p-ABS bindende Gruppe der Zelle während der aktiven Phase des normalen Stoffwechsels blockiert wird. O'Meara nimmt nun an, dass diese Blockierung am *Redukton* einsetzt. „If the place normally taken by p-aminobenzoic acid be occupied by a sulphonamide, cellular metabolism ceases."

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[1] Euler u. Hasselquist. Rec. Trav. Chim. Pays-Bas 69, 402 (1950). — Dieses Arkiv 2, Nr. 14 (1950).

[2] Angier et al. J. Am. Chem. Soc. 70 25 (1948).

[3] E. A. Bell, W. Cocker a. R. A. Q. O'Meara, Biochem. J. 45, 373 (1949). Die genannten Forscher geben für ihr Produkt den Schmpt 254° und die Zusammensetzung $C_{10}H_9O_4N.\ H_2O$ an und fanden einen N-Gehalt von 6,1 %.

[4] H. S. Forrest u. James Walker, Nature 161, 721 (1948). — J. Chem. Soc. (London) 1949, 97 u. 2003.

[5] D. D. Woods, Brit. J. exp. Pathol. 21, 74 (1940).

Die Konkurrenz zwischen p-ABS und Sulfonamiden hängt dann von der relativen Affinität der beiden Stoffe zum Redukton und von der rel. Löslichkeit der beiden Redukton-Derivate ab. O'MEARA et al. haben solche Versuche mit verschiedenen *Sulfonamiden* ausgeführt. Wir geben hier einen Versuch an, welcher sich auf das System *p-ABS-Sulfanilsäure* bezieht.[6]

Das zu diesen Versuchen erforderliche Kondensationsprodukt Redukton-Sulfanilsäure wurde folgendermassen dargestellt:

Sulfanilsäure und Redukton wurden im Verhältnis 1: 1 mit wenig Wasser erhitzt bis alles in Lösung ging. Zu der etwas abgekühlten Lösung wurde die 4-fache Menge Aceton zugegeben; nachdem sich die Lösung auf Zimmertemperatur abgekühlt hatte wurde abgesaugt und mit Äther gewaschen. Das so erhaltene Produkt wurde mit Aceton ausgekocht, um es von etwa noch vorhandenem Redukton zu befreien. Das gelb-orange gefärbte Sulfanilsäure-Redukton zersetzt sich bei schnellem Erwärmen bei etwa 300°. Die Substanz löst sich leicht in Wasser, schwer in Alkohol, fast gar nicht in Aceton und Äther.

In biochemischer Hinsicht ist der Befund von Interesse, dass eine *verdünnte, wässrige Lösung von Sulfanilsäure-Redukton schon durch kleine Mengen von neutralen Ca-Salzen gefällt wird.**

Eine 0,01 n. Lösung von Sulfanilsäure-Redukton ergibt durch Zusatz einer äquivalenten Menge $CaCl_2$ einen gelben, körnigen Niederschlag. Die Löslichkeit des Sulfanilsäure-Redukton-Ca-Salzes liegt unter 0,001 m.

Dieser Umstand ist beachtenswert für die Beurteilung von Sulfonsäure-Wirkungen auf Bakterien und Bakterien-Nährlösungen, welche Ca enthalten.

Unter unseren Versuchen über die Hemmung der Samen-Keimung sei hier folgender erwähnt:

Gerstensamen (Siegergeste, SVALÖF) wurden 14 St. in Leitungswasser und dann noch 10 St. in dest. Wasser unter Zusatz von 0,5 % $H_2O_2$ gequollen, dann in Krystallisierschaalen auf Filtrierpapier übergeführt, dem eine 0,3 %ige Lösung von Sulfanilsäure-Redukton (20 ml) zugesetzt war.

In den Parallelversuchen mit Wasser begann nach etwa 60 St. (20°) die Entwicklung von Keimlingen und Wurzeln, und zwar gleichheitlich bei allen Samenkörnern. Im Sulfanilsäure-Redukton-Versuch war die Keimung stark gehemmt; nach 5 Tagen begannen etwa $^1/_4$ der Samen zu keimen. Aus der Sulfanilsäure-Redukton-Lösung schied sich in den Krysttallisierschaalen ein Niederschlag ab, welcher als Ca-Fällung anzusprechen war. Etwa $^1/_3$ aller Samen verblieben nach 8 Tagen ungekeimt.

### Umsetzung von Sulfanilsäure-Redukton mit p-ABS

132 mg Sulfanilsäure-Redukton wurden mit 10 ml Wasser unter Stickstoff auf dem Wasserbad erwärmt, dazu wurden 70 mg p-ABS zugegeben. Die hellgelbe Farbe schlug fast augenblicklich in ein sattes Orange um, und es schied sich ein farbiges Produkt A ab, welches nach Abkühlung der Lösung abgesaugt, mit Wasser, Aceton und Äther gewaschen wurde. Ausbeute 118 mg. Um etwa noch vorhandene Sulfanilsäure zu entfernen wurde A mit 500 ml Wasser gekocht und von der siedend

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[6] Siehe hierzu R. KUHN u. K. SCHWARZ, Chem. Ber. 74, 1617 (1941), — E. F. MÖLLER u. K. SCHWARZ, Chem. Ber. 74, 1612 (1941).

*Anscheinend analoge Fällungen werden erzeugt durch Zusätze von Ba- und Cu-Salzen.

eissen Lösung abfiltriert. Ausbeute 25 mg. Die Titration mit NaOH ergab das quivalent Ä = 103. Für $C_{10}H_9O_4N$ ber. 104.

Sulfanilsäure-Redukton hat sich also mit p-ABS weitgehend zu p-ABS-Redukton nd Sulfanilsäure umgesetzt.

Die Schnelligkeit der Reaktion deutet darauf hin, dass es sich um den Ersatz der mino-Komponente in einer Schiff'schen Base handelt.

## p-ABS-Redukton und Sulfanilsäure

400 mg p-ABS-Redukton und 400 mf Sulfanilsäure wurden in Alkali gelöst und wa 10 Minuten stehen gelassen. Durch schwache Ansäuerung fiel eine Substanz ıs die abgesaugt und mit Wasser, Aceton und Äther gewaschen wurde. Die so haltene Substanz war gelb-orange gefärbt. Sie zersetzte sich bei etwa 260–264° e nach der Schnelligkeit der Erwärmung). Für unser reinstes p-ABS-Redukton-räparat hatten wir den Zersetzungspunkt 264° gefunden.

**Titration mit NaOH.** 3,8 mg Sbst. in 1 ml 0,11 n. NaOH gelöst und mit 0,11 n. Cl titriert. Verbrauch 0,68 ml HCl. Hieraus ergibt sich das Äquivalentgewicht = 3,8: (0,32 × 0,11) = 107,9. Ber. Ä = 104.

Es wurde also das angewandte p-ABS nach der Einwirkung der Sulfanilsäure un-erändert wiedergewonnen.

## Oxydation von p-ABS-Redukton

Das eingangs erwähnte Produkt, aus 1 Mol Redukton und 1 Mol p-ABS bestehend, elches die Endiolgruppe des Reduktons unverändert enthält, lässt sich, ebenso wie edukton selbst, durch Jod leicht oxydieren, und zwar ohne dass p-ABS abgespalten ird.

**Versuch:** 400 mg p-ABS-Redukton (HH 94) zusammen mit 1 g NaAc in 2 ml Alko-ol aufgeschlämmt, wurde mit 2 ml einer alkoholischen Lösung von 485 mg Jod rsetzt. Nach Ablauf der Reaktion (festgestellt mittels Stärkepapier) hatte sich das usgangsmaterial vollständig gelöst; es wurde nun mit dem gleichen Volumen Wasser rdünnt und mit 300 mg p-ABS versetzt. Nach $^1/_2$-stündigem Erwärmen auf dem edenden Wasserbad fielen aus der abgekühlten Lösung kleine rotbraune Krystalle ıs, welche abfiltriert und mit 50-%igem Alkohol gewaschen wurden.

Das Rohprodukt (350 mg) wurde mit Alkohol aufgekocht; die Lösung färbte sich n rotbraun, und die Hauptmenge der Krystalle blieb mit schmutziggelber Farbe gelöst zurück. Nach Waschen mit Alkohol schmolz das Produkt bei 307° unter rsetzung. Die Substanz HH 112 ist schwerlöslich in allen üblichen organischen sungsmitteln. Sie reduziert Tillmans-Reagenz nicht.

Das Produkt $C_{17}H_{12}O_5N_2$ dürfte demjenigen entsprechen, welches nach Angabe n Forrest und Walker[4] (Soc. 1949, 2002) aus Redukton und p-ABS entsteht. zeigt sich nun, dass diese Substanz das Kondensationsprodukt aus 1 Mol Mesoxal-aldehyd und 2 Mol p-ABS darstellt.

p-Aminobenzoesäure ist als ausserordentlich wirksamer Bakterienwuchsstoff vor Jahren durch Woods[5] sowie Rubbo und Gillespie[7] nachgewiesen worden. Etwa gleichzeitig ergab sich der Antagonismus p-ABS-Sulfonamide bezw. p-ABS-Sulfanilsäure sowie die Auffassung dieses Antagonismus als Konkurrenz an einer den Stoffwechsel der Zelle vermittelnden Gruppe (Fildes; R. Kuhn).

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[7] S. D. Rubbo a. J. M. Gillespie, Nature 146, 838 (1940).

1944 stellte O'Meara, wie schon erwähnt, die Hypothese auf, dass die p-Aminobenzoesäure in der wachsenden Bakterienzelle an das Redukton gebunden wird und in dieser Bindung als Wuchsstoff fungiert.

Am Redukton soll die Konkurrenz zwischen p-ABS und Sulfonamid stattfinden.

Nun ist p-ABS-Redukton Ausgangsmaterial für die als Bakterienwuchsstoff fungierende *Folinsäure.* Ohne auf die ausserordentlich reichhaltige Litteratur über Folinsäure, Vitamin $B_{12}$, L. casei Faktor u. s. w. einzugehen, sei hier nur an Folgendes erinnert:

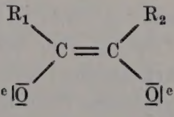
Tschesche[8] hat 1947 beobachtet, dass die Bildung von Folinsäure in Mikroorganismen durch Sulfonamide gehemmt wird. Diese Hemmung der Folinsäure-Synthese durch Sulfonamide *erfolgt via p-ABS,* wie durch Versuche von Lampen und Jones[9] sowie von Möller, Weygand und Wacker[10] gezeigt wird. Besonders bemerkenswert ist der Befund von Auhagen[11] über das verschiedene Verhalten gewisser Bakteriengruppen zu Folinsäure bezw. p-Aminobensoesäure und zu Sulfonamiden:

Bakterien der Gruppe A wachsen in folinsäurefreier Nährlösung und werden durch Sulfonamide nicht gehemmt;

Auch die Bakterien der Gruppe B wachsen in folinsäurefreiem Medium, sind aber durch Sulfonamide hemmbar;

Bakterien der Gruppe C wachsen nur bei Anwesenheit von Folinsäure; sie werden durch Sulfonamide nicht gehemmt.

Die Reduktone sind zunächst durch ihre ausserordentlich hohe Reduktionsfähigkeit charakterisiert. Dieselbe kann darauf zurückgeführt werden, dass der oxydierende Stoff dem doppelt gebundenen C-Atompaar I der Endiolgruppe 2 Elektronen ($\pi$-Elektronen) entzieht, wodurch das Biradikal II entsteht. Dieses geht dann durch eine einfache Elektronen-Verschiebung in die Diketogruppe III über.



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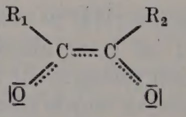

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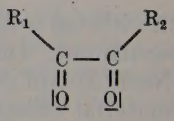

III

Die erwähnte Elektronen-Verteilung in den Endiolgruppen macht sich bei den verschiedenen Reduktonen in verschiedener Weise geltend, und zwar nicht nur bei den Oxydo-Reduktions-Vorgängen, sondern auch bei anderen Reaktionen (Tabelle 1) auf die wir in einer späteren Mitteilung noch zurückkommen. Hier sei nur kurz an die Analogien erinnert, welche in biochemischer Hinsicht zwischen dem Triose-Redukton und den beiden anderen Reduktonen Ascorbinsäure und Brenzcatechin hinsichtlich ihres Verhaltens zu $H_2O_2$ und Peroxydase bestehen.[12]

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[8] Tschesche, Z. f. Naturforsch. 2 b, 10 (1947).

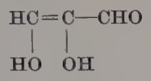
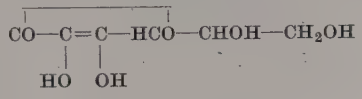
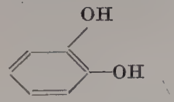
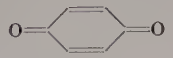
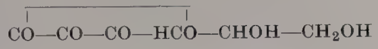
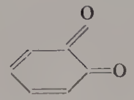
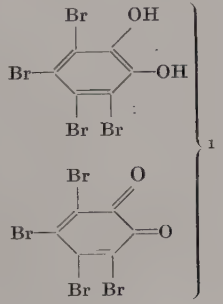
[9] J. O. Lampen u. M. J. Jones, J. biol. Chem. 164, 485 (1946). — 166, 435 (1946).

[10] E. F. Möller, F. Weygand u. A. Wacker, Z. f. Naturforsch. 4 b, 100 (1949) 5 b, 18 (1950). Diese Forscher stellten u. a. fest, dass Sulfonamide bei der Gruppe B ausser durch Folinsäure auch von verschiedenen Derivaten der Folinsäure, von Thymin sowie von pABS und von p-Amino-benzoyl-l-glutaminsäure enthemmt werden.

[11] E. Auhagen, Z. physiol. Chem. 183, 195 (1948).

[12] Es sei besonders auf die Arbeiten von Szent-Györgyi und seiner Schule hingewiesen.

*Tabelle 1*

| Reagenz | Triose-Redukton | Ascorbinsäure | Brenzcatechin | p-Chinon |
|---|---|---|---|---|
| |  $\underset{\text{HO}}{\text{HC}}=\underset{\text{OH}}{\text{C}}\text{—CHO}$ |  $\overline{\text{CO—}\underset{\text{HO}}{\text{C}}=\underset{\text{OH}}{\text{C}}\text{—HCO}}\text{—CHOH—CH}_2\text{OH}$ |  |  |
| Tillmans Reagenz (TR) | CHO—CO—CHO |  $\overline{\text{CO—CO—CO—HCO}}\text{—CHOH—CH}_2\text{OH}$ |  | $+2\,H_2O$ in alkal. Lös.<br>Entfärbung entspr. 2 Endiolen |
| $Br_2$ | Entfärbung unter Bildung von HBr | » |  [1] | |

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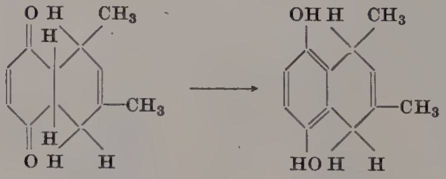
[1] Siehe Th. Zinke, Ber. 20, 1776 (1887).

Von besonderem Interesse ist aber der Vergleich zwischen der Elektronen-Verteilung einerseits in den Endiolen, andererseits in Atomanordnungen vom Typus —CO—CH = CH—CO—, wie sie in Chinonen und in manchen anderen dienophilen Substanzen vorkommen. Probleme, welche sich aus dem Studium der Reduktone ergeben, haben uns veranlasst, den Mechanismus gewisser *Diensynthesen* näher zu verfolgen, und damit in ein früheres Forschungsgebiet unseres Arbeitskreises zurückzukehren.

1920 hatten EULER und JOSEPHSON[13] eine Kondensation von Isopren und Chinon durchgeführt und diese Reaktion als Diensynthese beschrieben. Seit etwa 1928 sind dann viele hunderte von Diensynthesen ausgeführt worden; besonders verdankt man DIELS und ALDER ein ausserordentlich grosses und vielseitiges experimentelles Material; als Philodien kam dabei überwiegend Maleinsäure-Anhydrid zur Verwendung. Die Diensynthesen sind in letzter Zeit wiederholt zusammengefasst worden (Neuere Methoden der präparativen organ. Chemie. Verlag Chemie 1943. — Organic Reactions. Vol. IV. New York; London 1948), sodass es sich erübrigt, diesen Reaktionstypus als solchen zu besprechen. Dagegen wollen wir hier, z. T. in Anschluss an eine vorhergehende Mitteilung[14], auf einige Resultate eingehen, welche sich bei unseren neueren Dienarbeiten ergeben haben.

## B. Umlagerungen an Dien-Derivaten

In der vorhergehenden Arbeit[14], welche an die Umlagerung des aus 1,3-Dimethylbutadien und p-Chinon gewonnenen Reaktionsproduktes HH 105 in die Substanz HH 106 anknüpft, wurden einige vorläufige Versuche über die dabei eintretende Wärmeentwicklung beschrieben. Wir kommen nun auf diesen interessanten, durch HBr katalysierten Vorgang und die Wärme-Entwicklung des ausserordentlich energiereichen Typus 105 zurück.



105 106

Zunächst wurde festgestellt, dass der Katalysator HBr, der bei der genannten Umlagerung in sehr geringer Konzentration verwendet wurde, durch starke Erhöhung der Konzentration nicht oder nur unwesentlich an Wirksamkeit zunimmt. Er kann nicht durch Schwefelsäure ersetzt werden (es liegt also keine allgemeine pH-Wirkung vor), wohl aber durch HCl, welches sich ebenfalls an die Carbonylgruppe des Chinons unter Bildung eines Oxoniumsalzes anlagert.[15]

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[13] EULER u. K. JOSEPHSON, Chem. Ber. 53, 822 (1920).

[14] EULER u. H. HASSELQUIST, Dieses Arkiv 2, N. 23 (1950).

[15] Vgl. B. EISTERT, Chemismus und Konstitution. 1. Band, S. 232. — Siehe hierzu auch die Bemerkung von DIELS und ALDER, Ber. 62, 2337 u. zw. 2346 (1929). — Nach dem gegenwärtigen Stand der chemischen Elektronentheorie (B. EISTERT) wird „infolge des bei der Oxoniumsalzbildung auftreten den starken Dipols das anguläre H-Atom als Proton abgestossen, worauf das Bindungssystem in das der Enolform umklappt“.

Auch die Säurekatalyse von Hydrolysen wird durch intermediäre Bildung eines Oxoniumsalzes vermittelt.

Die Probleme, denen wir uns, von obigem Reaktionstypus ausgehend, zugewandt haben, betreffen die Wärmeentwicklung, die — im Specialfall des 1,3-Dimethylbutadien-Chinons — bei der Bildung der Substanz 105 und bei deren Übergang in die Substanz 106 eintreten. Wir wollen im Folgenden die beiden Substanztypen mit N I und N II bezeichnen. Es handelt sich zunächst um die Grösse der entwickelten Wärmemengen und um ihre Beziehung zur Änderung der Stabilität und zur Abnahme der chemischen Energie.

Das zweite, viel umfassendere Problem betrifft die bis jetzt noch ganz unbekannte und wenig diskutierte Ausbildung eines Wärme-Effektes auf Kosten einer mit Abnahme der chemischen Energie verbundenen Atomverschiebung. Wir wollen auf dasselbe in späteren Mitteilungen eingehen.

## Wärme-Entwicklung beim Übergang der 1,3-Dimethyl-butadien-Derivate HH 105 in HH 106

Vor kurzer Zeit (dieses Arkiv 2, Nr. 23) haben wir die Temperatur-Steigerung angegeben, welche in der dort beschriebenen primitiven Anordnung am System HH 105-Eisessig-HBr abgelesen worden war. Die mit dieser Anordnung erreichte höchste Temperaturerhöhung entspricht, wie l. c. schon betont wurde, nicht der entwickelten Wärmemenge, sondern ist durch den Wärmeverlust, den das System während des Versuchs durch Ausstrahlung erfährt, wesentlich vermindert.

Für diesen Wärmeverlust kann mit einiger Annäherung eine Korrektion eingeführt werden.

Aus der experimentell ermittelten Abstrahlungskurve kann ein mittlerer Wärmeverlust von 2,9° per Minute entnommen werden. Nach etwa 3 Minuten stimmte der Verlauf der Abstrahlungskurve mit dem der Umlagerungskurve überein; die Temperatur betrug dann 44,8°.

Die Temperatur, welche ohne Wärmeverlust des Systems erreicht worden wäre, lässt sich zu $44{,}8 + 3 \times 2{,}9 = 53{,}5°$ berechnen.

Bei Beginn der Reaktion lag die Temperatur bei 22,6°; also betrug die der gesammten Reaktionswärme entsprechende Temperaturerhöhung $53{,}5 — 22{,}6 = 30{,}9°$.

Der Wasserwert des Systems liess sich angenähert zu 2,1 cal ermitteln. Dann ergibt sich für 600 mg der Substanz 105 die entwickelte Wärmemenge zu 65,2 cal. Für 1 Mol der Substanz 105 berechnet sich die Umwandlungswärme zu rund 21 K cal. Dabei wird angenommen, dass die Umwandlung 105–106 praktisch vollständig verläuft, wofür die chemische Untersuchung des Endproduktes einen ausreichenden Anhaltspunkt lieferte.

Das Studium der atomaren und elektronischen Vorgänge bei Übergängen von labilen in stabile Anordnungen vom Typus der oben erwähnten Reaktion 105–106 macht eine spektrophotometrische Untersuchung der Feinstruktur wünschenswert. Orienterende Aufnahmen sind zunächst zum Vergleich der Spektren der Substanzen 105 und 106 gemacht worden; sie werden später näher besprochen werden. Hier sei nur noch auf die Analogien hingewiesen, welche sich mit den eingehenden Absorptionsmessungen von C. J. P. Spruit[16] ergeben.

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[16] C. J. P. Spruit, Rec. Trav. Chim. Pays-Bas, 68, 309 (1949).

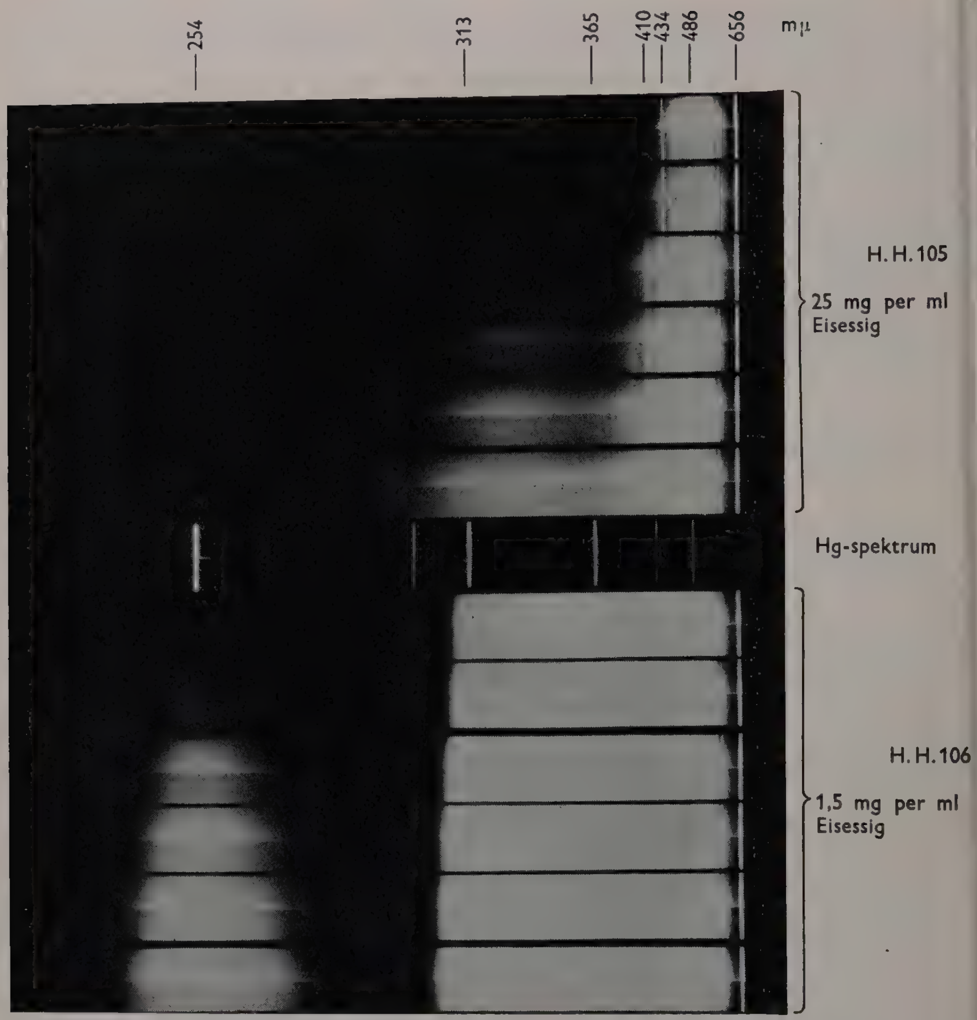

Fig. 1. Spektralaufnahme der Subst. H.H. 105 und H.H. 106 mit $H_2$-Lampe.

## Wärme-Entwicklung bei der Synthese der Substanz HH 105

Auch bei der *Bildung* der Substanz HH 105 tritt eine erhebliche Wärme-Entwicklung ein, und es war naheliegend, eine Beziehung derselben zu der Wärmetönung der Umlagerung 105–106 zu suchen. Indessen konnte die bei der Synthese der Substanz 105 auftretende Wärmemenge noch nicht gemessen werden.* In diesem Zusammenhang ist folgendes zu erwähnen:

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* Es soll versucht werden, das Ziel unter Verwendung anderer Diene zu erreichen.

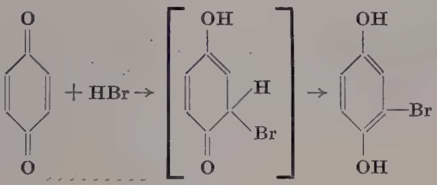
Die hier verwendeten sowie eine Reihe weiterer Diene verdanken wir Herrn Direktor Dr. Dr. h. c. WALTER REPPE, Badische Anilin- und Soda-Fabrik Ludwigshafen/Rhein.

I. Mischt man äquivalente Mengen von p-Chinon und 1,3-Dimethyl-butadien und erwärmt die Mischung auf 70° (also auf eine Temperatur unterhalb des Siedepunktes des Butadiens), so krystallisiert nach Abkühlen der Mischung das Chinon unverändert aus. Das Umwandlungsintervall ist nicht erreicht.

Lässt man dagegen die Temperatur bis zum Siedepunkt des 1,3-Dimethyl-butadiens (76°) steigen, so setzt sofort eine rasch verlaufende Reaktion ein, und die mit derselben verbundene Wärme-Entwicklung hält das Reaktionsgemisch (ohne äussere Wärmezufuhr) im Sieden, bis sich alles Ausgangsmaterial in die Substanz 105 umgesetzt hat. Eine Anfangstemperatur von gewisser Höhe ist also erforderlich.

II. Wenn die unter I angegebene Reaktionsmischung von Anfang an mit wenigstens so viel HBr versetzt wird, als zur katalytischen Umwandlung in 106 verwendet wurde, so tritt eine Bildung von 106 *nicht* ein, sondern die Reaktion bleibt, wie ohne HBr, bei der Substanz 105 stehen.

Dass hier HBr nicht umlagernd in Wirkung tritt dürfte sich folgendermassen deuten lassen: In Gegenwart von unverändertem p-Chinon reagiert HBr mit diesem und wird dadurch aus dem System entfernt:



Das so gebildete Brom-hydro-chinon kann mit dem Dien nicht reagieren da HBr hier nicht abgespalten werden und freies HBr und Chinon bilden kann. Auch wenn man dem Reaktionsgemisch von Anfang an so viel HBr zusetzen würde, dass freies HBr vorhanden ist, so würde dadurch auch die Bildung von Subst. 105 verhindert.

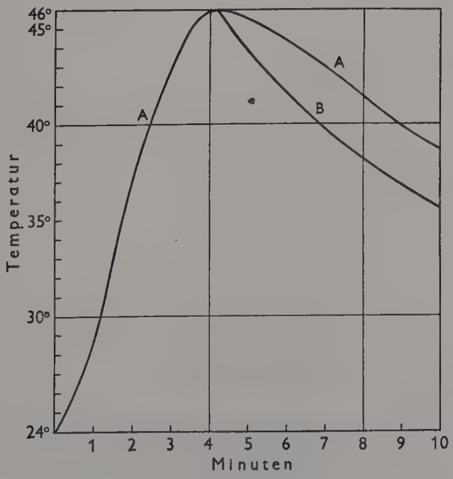


Fig. 2.

## Wärme-Entwicklung beim Übergang der Isopren-Derivate I und II[17]

In der früher verwendeten Versuchsanordnung wurden 555,2 mg Isopren-Chinon[17] in 2 ml Eisessig gelöst und auf 24,1° abgekühlt. Zu dieser Lösung wurde ein Tropfen HBr-Eisessig-Lösung gegeben. Die sofort eintretende Temperatur-Steigerung wurde unter Umrühren mit einem $^1/_{10}$° geteilten Thermometer verfolgt. Bei 44°–45° fiel der grösste Teil des Reaktionsproduktes aus.

Die beobachtete Wärme-Entwicklung ergibt sich aus der obigen Figur 2. Wie aus derselben hervorgeht, tritt die Temperatursteigerung beim Isopren-Derivat etwas langsamer ein als bei der Substanz 105, der Verlauf ist aber ein ähnlicher und die entwickelte Wärmemenge ist von der gleichen Grössenordnung.

Stockholm, Universität, Vitamin-Institut und Inst. für organ.-chem. Forschung.

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[17] In einem Referat zweier Patentschriften der I. G. Farbenindustrie A. G. (E. P. 324 661 vom 26/9 1928 und F. PP. 677 296 und 677 781, 1929) im Chem. Zbt. 101, II 809; (1930) ist erwähnt, dass man durch Kondensation von Benzochinon mit Isopren bei 50° unter Druck eine Verbindung erhält, die in der 7-Stellung eine Methylgruppe enthält. Schmelzpunkt und sonstige Eigenschaften sind nicht angegeben.

Wir haben Isopren und Chinon im Verhältnis 1:1 in Benzol unter Rückfluss 2 St. zum Sieden erhitzt. Nach dem Einengen erstarrte das Reaktionsmasse beim Anreiben. Nach dem Umkrystallisieren aus Petroläther schmolzen die leicht grünlich gefärbten Krystalle bei 84°–85°.

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Mitgeteilt am 25. Oktober 1950 von H. v. EULER

# Papierchromatographische Untersuchung der Reduktonbildung.

Von FRIEDRICH WEYGAND

Seit der Einführung der Papierchromatographie durch R. CONSDEN, A. H. GORDON und A. J. P. MARTIN[1] ist dieses elegante Verfahren zur Trennung von Aminosäuren, Zuckern, Pyrimidinen, Purinen und anderen Verbindungen herangezogen worden. Auch zum Studium der *Reduktonbildung* aus Zuckern unter den von H. VON EULER und Mitarbeitern[2] angegebenen Bedingungen (Erhitzen in verdünnter Natronlauge), sowie zur Unterscheidung von Redukton, Ascorbinsäure, Cystein und Glutathion und Prüfung der Frage, ob Redukton von Bakterien gebildet wird (R. A. Q. O'MEARA und Mitarb.[3]), schien die Papierchromatographie die gegebene Methode zu sein.

## Nachweis von Redukton und Ascorbinsäure auf Papierchromatogrammen

Redukton (Enol-tartronaldehyd) und Ascorbinsäure zeichnen sich dadurch aus, dass sie bereits in saurer Lösung Silbernitrat reduzieren und *Tillmanns*-Reagens (Dichlorphenol-indophenol) entfärben. Redukton reagiert ferner mit p-Aminobenzoesäure unter Bildung eines gelben Kondensationsproduktes[3, 4, 5], während das mit Ascorbinsäure nicht der Fall ist. Diese Reaktionen können zur Erkennung und Unterscheidung von Redukton und Ascorbinsäure und anderen Endiolen auf Papier benutzt werden. Redukton und Ascorbinsäure zeigen ferner stark verschiedene $R_F$-Werte bei Verwendung eines Entwicklungsgemisches aus n-Butanol-Eisessig-Wasser (vergl. Tabelle 1).

Als Entwicklungsflüssigkeit diente die obere Phase eines aus 100 Vol. n-Butanol, 20 Vol. Eisessig und 50 Vol. Wasser hergestellten Gemisches oder die homogene Mischung aus 100 Vol. n-Butanol, 20 Vol. Eisessig und 30 Vol. Wasser.

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[1] Biochem. J. *38*, 224 (1944).

[2] H. VON EULER u. E. KLUSSMANN, dieses Arkiv *11 B*, Nr. 7 (1933). H. VON EULER u. C. MARTIUS, Ann. *505*, 73 (1933). H. VON EULER u. H. HASSELQUIST, Reduktone, Verlag F. Enke, Stuttgart 1950.

[3] R. A. Q. O'MEARA, P. A. MCNALLY, H. G. NELSON, Nature, London, *154*, 796 (1944). Lancet 1947, II, 747. E. A. BELL, W. COCKER u. R. A. Q. O'MEARA, Lancet 1948, I, 924, Biochem. J. *45*, 373 (1949). W. COCKER, R. A. Q. O'MEARA, J. C. P. SCHWARZ u. E. R. STUART, J. Chem. Soc. *1950*, 2052.

[4] R. B. ANGIER et al., J. Amer. chem. Soc. *70*, 25 (1948). H. S. FORREST u. J. WALKER, Nature, London, *161*, 721 (1948), J. chem. Soc. *1949*, 79, 2002.

[5] H. VON EULER, H. HASSELQUIST u. U. LÖÖV, dieses Arkiv *26 A*, Nr. 17 (1948). H. VON EULER u. H. HASSELQUIST, Rec. *69*, 402 (1950).

Es wurden *Whatman*-Papier Nr. 1 oder *Munktell OB* verwendet, von denen sich das letztere durch grössere Sauggeschwindigkeit auszeichnet. Die Papierchromatographie wurde unter grossen Glasglocken ausgeführt, wobei zum Schutze der reduzierenden Substanzen jeweils beim Beginn der Versuche aus Natriumsulfid und Essigsäure Schwefelwasserstoff in geringem Umfange entwickelt wurde. Nach Beendigung der Chromatogramme wurden die Papiere im Abzug etwa 15 Min. lang zum Trocknen aufgehängt bevor sie mit den Nachweisreagentien besprüht wurden.

Von den in Tabelle 1 aufgeführten Substanzen wurden je 0,005 bis 0,01 ccm auf die Startlinie der Chromatogramme gebracht. Entwicklungszeit 4 bis 16 Stunden bei 19 bis 22°.

*Tabelle 1.*

| | Redukton | Ascorbinsäure | Dioxyaceton |
|---|---|---|---|
| $R_F$-Werte | 0,59 bis 0,64 | 0,31 bis 0,36 | 0,39 bis 0,43 |
| *Nachweis-Reagens* | | | |
| 0,001-n Tillmanns-Reagens in Wasser | Entfärbung | Entfärbung | — |
| 1-proz. p-Aminobenzoesäure in 10-proz. Essigsäure | Gelbfärbung[6] | — | — |
| 1-proz. Silbernitrat in Wasser | Schwarzfärbung | Schwarzfärbung | — |
| 1-proz. Silbernitrat in Ammoniak | Schwarzfärbung | Schwarzfärbung | — |
| 1-proz. Silbernitrat in Ammoniak + gleiches Vol. 2-n Natronlauge | Schwarzfärbung | Schwarzfärbung | Schwarzfärbung |

$R_F$-Werte von Redukton, Ascorbinsäure und Dioxyaceton und ihre Nachweismöglichkeiten auf Papier. Entwicklungsgemisch: Obere Phase einer Mischung aus 100 Vol. n-Butanol, 20 Vol. Eisessig und 50 Vol. Wasser. Die Sichtbarmachung der Flecken geschah mit den aufgeführten Reagentien bei Zimmertemperatur.

Gewisse Kondensationsprodukte von Redukton mit Aminen, wie mit Anilin, N-Methyl-anilin, p-Aminobenzoesäure oder N-Methyl-p-aminobenzoesäure[7] zeigen bei der Papierchromatographie eine Aufspaltung in das Amin und Redukton, das einen Fleck mit dem richtigen $R_F$-Wert liefert. Die Kondensationsprodukte waren in 50-proz. Ameisensäure gelöst und mit dem Butanol-Essigsäure-Wasser Gemisch chromatographiert worden. Das Kondensationsprodukt aus Redukton und Sulfanilsäure zeigte diese Aufspaltung in die Komponenten nicht. Es teilte sich aber in zwei Flecke mit den $R_F$-Werten 0,21 (stark gelb) und 0,37 (ganz blass gelb) auf.

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[6] Das Kondensationsprodukt aus Redukton und p-Aminobenzoesäure zeigt im Gegensatz zu den Angaben verschiedener Autoren keine grüne Fluorescenz.

[7] Aus 1 g N-Methyl-p-aminobenzoesäure in 40 ccm Wasser + 20 ccm Alkohol + 4 ccm Eisessig (bei 55° gelöst), + 1 g Redukton. Nach 24 Stdn. wurde abgesaugt. Ausbeute 1,3 g. Nach Umkristallisieren aus 50-proz. Essigsäure blassgelbe feine Nädelchen vom Schmp. 232°. $C_{11}H_{11}O_4N$ (221,1.) Ber. C 59,72 H 5,02 N 6,33, Gef. C 59,68 H 5,01 N 6,35.

### Reduktonbildung aus verschiedenen Zuckern

Redukton wird gewöhnlich nach der Vorschrift von EULER und MARTIUS[2] aus Glukose durch Erwärmen mit Natronlauge über das Bleisalz gewonnen. Was nun die Reduktonbildung aus anderen Zuckern betrifft, so ist bereits von EULER und HASSELQUIST[8] gezeigt worden, dass sich auch aus Dioxyaceton beim Erwärmen mit Natronlauge Redukton bildet: Es wurde der Di-p-nitrobenzoesäureester gewonnen. Ferner konnte Herr Armin GLASER in diesem Institut aus d-Xylose eine geringe Menge Redukton als p-Nitranilin-Redukton-Verbindung isolieren.

Es wurde nun mit Hilfe der papierchromatographischen Technik geprüft, ob auch aus anderen Zuckern Redukton entsteht. Bei der ersten Versuchsreihe wurde die übliche Bleisalzfällung mitverwendet, während diese bei der zweiten Reihe weggelassen wurde.

Je 10 mg von Glukose, Fruktose, Arabinose und Rhamnose wurden in 0,5 ccm Wasser mit 6 mg Bleiacetat auf 90° erwärmt. Dann fügte man unter Einleiten von Stickstoff 0,2 ccm 1-n Natronlauge, die Spuren von KCN enthielt, hinzu und hielt 3 Min. lang bei 90–92°. Nach dem schnellen Abkühlen fügte man 10-proz. Essigsäure hinzu bis ein Niederschlag ausfiel ($p_H = 6{,}5$–$7$). Das Bleisalz wurde abzentrifugiert, mit einigen ccm Wasser gewaschen und nach Suspendierung in Aceton mit Schwefelwasserstoff zerlegt. Man zentrifugierte das Bleisulfid ab, goss die Acetonlösung in ein kleines Reagenzglas und verjagte das Aceton mit Stickstoff. Dann fügte man 2 Tropfen Wasser hinzu und prüfte nun, ob sich papierchromatographisch Redukton nachweisen liess. Es ergab sich, dass aus den erwähnten vier Zuckern Redukton gebildet worden war. Mit *Tillmanns*-Reagens, p-Aminobenzoesäure oder Silbernitratlösung liess sich jeweils nur 1 Fleck mit dem für Redukton characteristischen $R_F$-Wert nachweisen.

Es wurde sodann untersucht, ob neben Redukton noch andere stark reduzierende Verbindungen beim Erwärmen der Zucker mit Alkali entstehen. Bei der soeben wiedergegebenen Versuchsreihe war eine Bleisalzfällung vorgenommen worden. Diese wurde nun weggelassen, da im Bleisalz ausser Redukton keine anderen Verbindungen gefunden worden waren, die in saurer Lösung *Tillmanns*-Reagens zu reduzieren vermögen.

Zur Untersuchung kamen folgende Zucker: Dioxyaceton, Arabinose, Ribose, Glukose, Galaktose, Fruktose, Rhamnose, Maltose, Laktose sowie Rohrzucker und Raffinose. Je 10 mg Zucker wurden unter Stickstoff mit 0,2 ccm 1-n Natronlauge, die Spuren von KCN enthielt, 3 Min. lang auf 90° erwärmt. Die verschlossenen Versuchsröhrchen wurden vollständig in das heisse Wasser eingetaucht, damit kein Wasser verdampfen und sich so etwa die Konzentration der Lauge erhöhen konnte. Die alsdann schnell auf 20° abgekühlten Röhrchen wurden mit 1 Tropfen Eisessig angesäuert.

Zum Nachweis der entstandenen reduzierenden Substanzen wurden drei Chromatogramme angesetzt, die a) mit *Tillmanns*-Reagens, b) mit p-Aminobenzoesäurelösung und c) mit wässriger Silbernitratlösung besprüht wurden. Es ergab sich, dass aus allen untersuchten Zuckern mit Ausnahme von Rohrzucker und Raffinose, denen die zur Einleitung der Reduktonbildung notwendige freie Aldehyd- oder Ketogruppe fehlt, Redukton gebildet worden war. Mit p-Aminobenzoesäurelösung besprüht ergaben die Papiere nur gelbe Flecke bei dem für Redukton charakteristischen $R_F$-Wert. Durch Besprühen mit *Tillmanns*-Reagens oder wässriger Silbernitratlösung wurde jedoch erkannt, dass ausser Redukton noch andere, in saurer Lösung stark reduzierende Substanzen bei der Alkalibehandlung der Zucker in der Hitze entstehen. Zwei dieser Verbindungen wandern schneller als Redukton und hatten $R_F$-Werte von 0,85 und 0,92, die langsamer wandernden zeigten $R_F$-Werte von $0{,}27_8$ und $0{,}34_4$. Redukton selbst hatte bei diesem Versuch den $R_F$-Wert von 0,68 (Entwicklungsgemisch: 100 Vol. n-Butanol, 20 Vol. Eisessig und 30 Vol. Wasser).

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[8] Dieses Arkiv *26 A* Nr. 25 (1949).

Über die Natur der ausser Redukton bei der Alkalibehandlung der Zucker in der Hitze noch entstehenden stark reduzierenden Verbindungen können im Augenblick nur Vermutungen angestellt werden. Nach der Grösse der bei der Papierchromatographie auftretenden Flecken zu urteilen, entstehen diese Verbindungen in ähnlichen Mengen wie Redukton selbst. Es ist nicht ausgeschlossen, dass es sich bei den langsamer als Redukton wandernden Verbindungen um Endiole handelt, bei denen die Doppelbindungen zwischen den C-Atomen 2,3 oder 3,4 der Zuckerkette liegt. Derartige Verbindungen würden sich von 3-Ketozuckern ableiten und vielleicht sind deren Endiole auch in saurer Lösung stabil, da sie von C-Atomen umgeben sind, die Sauerstoffatome in Form von Hydroxylgruppen tragen. Die von Aldosen oder 2-Ketozuckern abgeleiteten 1,2-Endiole scheinen sich nach unseren bisherigen Kenntnissen in saurer Lösung leicht in die Aldosen und 2-Ketozucker zurückzuverwandeln. Im übrigen unterscheiden sich 2-Ketohexosen und 2-Ketopentosen durch die Leichtigkeit, mit der sie in alkalischer Lösung enolisieren. So kann Fruktose nach WILLSTÄTTER-SCHUDEL nicht mit Hypojodit titriert werden, aber die d-Xyloketose ist nach O. TH. SCHMIDT und R. TREIBER[9] unter den Bedingungen der WILLSTÄTTER-SCHUDEL Titration oxydierbar, d. h. sie enolisiert viel leichter als Fruktose.

## Nachweis von Ascorbinsäure in Naturprodukten

Mit Hilfe der Papierchromatographie lässt sich leicht entscheiden, ob neben Ascorbinsäure in natürlichen Materialien noch andere stark reduzierende Verbindungen vorkommen. In diesem Zusammenhang interessiert insbesondere auch die Frage, ob Redukton (Enol-tartronaldehyd) in der Natur gebildet wird.

So wurde zunächst gefunden, dass in frisch ausgepresstem *Orangensaft* Ascorbinsäure die alleinige Substanz ist, die *Tillmanns*-Reagens reduziert.

Als zweites Versuchsobjekt dienten die Augenlinsen von *Rindern*, deren Ascorbinsäuregehalt schon früher wiederholt bestimmt worden ist.[10] Es wurden z. B. 0,4 mg Ascorbinsäure/g Linse titrimetrisch ermittelt.[10] Durch Tierversuche konnte durch H. VON EULER[11] das Vorkommen von Ascorbinsäure bestätigt werden, was von Bedeutung war, da die übliche Titration mit *Tillmanns*-Reagens selbst bei pH-2,5 noch Cystein erfasst[12]. Andererseits waren die Tierversuche on EVERETTE und EVANS mit Augenlinsen negativ.[13] Der eventuelle chromato-raphische Nachweis von Ascorbinsäure in Augenlinsen schien daher von Inte-esse zu sein.

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[9] Ber. *66*, 1765 (1939).

[10] B. VON EULER u. H. VON EULER, Svensk Kemisk Tidskrift *45*, 173 (1933). H. VON EULER u. M. MALMBERG, Archiv für Augenheilkunde *109*, 225 (1935).

[11] Svensk Kem. Tidskr. *46*, 201 (1934). Vergl. auch BIRCH und DANN, Biochem. J. *28*, 638 (1934).

[12] C. MARTIUS u. H. VON EULER, Biochem. Z. *271*, 9 (1934), Hoppe-Seylers Z. *222*, 65 (1933).

Zwei Augenlinsen wurden in einem Mörser fein zerrieben, worauf man einige cm Schwefelwasserstoffwasser zum Schutze der reduzierenden Substanzen zufügte. ann verrührte man mit 20 bis 30 ccm 96-proz. Alkohol, filtrierte von der aus-efallenen Masse ab und wusch diese mit Alkohol nach. Nun fügte man zwei ropfen Eisessig hinzu und dampfte im Vacuum bei 30 bis 40° unter Einleiten on Stickstoff fast zur Trockne. Nach Verrühren mit 0,5 ccm Schwefelwaser-toffwasser setzte man Papierchromatogramme an, wobei auf die geringen Reste ngelöster weisser Masse keine Rücksicht genommen wurde. Durch Parallel-hromatogramme mit Ascorbinsäure ergab sich, dass diese tatsächlich in Rinder-nsen enthalten ist ($R_F$-Wert von synth. Ascorbinsäure $0{,}37_5$ und der Ascorbin-iure im Linsenextrakt $0{,}37_5$). Daneben trat beim Besprühen mit *Tillmanns*-eagens noch eine weitere reduzierende Verbindung mit einem geringeren $R_F$-Vert auf, die *Tillmanns*-Reagens erst im Verlaufe von einigen Minuten redu-ierte. Die Reduktion durch Ascorbinsäure oder auch durch Redukton erfolgt agegen momentan. Redukton konnte im Linsenextrakt ebenso wenig wie im rangensaft gefunden werden.

Um zu entscheiden, ob es sich bei der *Tillmanns*-Reagens langsamer redu-ierenden Verbindung um Cystein oder Glutathion handelt, wurden Parallel-hromatogramme mit diesen beiden Verbindungen angesetzt. Cystein zeigte mit em Butanol-Essigsäure-Wasser Gemisch (100 : 20 : 30) einen $R_F$-Wert von 0,24 nd Glutathion von 0,18. Beide Verbindungen reduzieren *Tillmanns*-Reagens auf em Papier erst im Verlaufe von einigen Minuten. Die langsamer wandernde ubstanz aus den Augenlinsen von Rindern zeigte nun den $R_F$-Wert von 0,17, t also mit Glutathion identisch. Wenn Cystein überhaupt vorhanden ist, so t seine Menge sehr viel geringer als die von Glutathion. Bei diesen Versuchen urde wie üblich etwas Schwefelwasserstoff im Gasraum entwickelt. Der starke lutathionfleck konnte auch durch Besprühen mit Ninhydrin und Erhitzen auf 00° sichtbar gemacht werden.

## Zur Frage der Reduktonbildung durch Bakterien

Von O'MEARA und Mitarb.[3] ist die Vermutung ausgesprochen worden, dass akterien in der Phase des logarithmischen Wachstums Redukton (Enol-tartron-ldehyd) bilden. Dies wurde durch die in sodaalkalischer Lösung erfolgende eduktion von o-Dinitrobenzol gefolgert, wobei eine Violettfärbung auftritt.[14]

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[13] Nature, London, *134*, 180 (1934).

[14] Es sei in diesem Zusammenhang darauf hingewiesen, dass die Reduktion von o-Dini-obenzol durch Ascorbinsäure oder Redukton zuerst von R. KUHN und F. WEYGAND (Ber. , 1969 (1936)) beschrieben worden ist und nicht von W. R. FEARON und E. KAWERAU iochem. J. *37*, 326 (1943)). Den zuletzt genannten Autoren ist die Arbeit von Kuhn und eygand offenbar entgangen, da sie für das violette Reduktionsprodukt noch eine falsche, üher angenommene Formulierung angeben. In Wirklichkeit handelt es sich um das aci-itrosalz von o-Nitro-phenylhydroxylamin, das selbst auch in kristallisierter Form isoliert urde. Das gleiche Verhalten wie o-Dinitrobenzol gegen Ascorbinsäure oder Redukton zeigt e 3,4-Dinitrobenzoesäure (F. WEYGAND u. H. HOFMANN, Ber. *83*, 405 (1950)), die zu 2-itro-4-carboxy-phenylhydroxylamin reduziert wird, dessen aci-Nitrosalz ebenfalls violett ist.

In einigen orientierenden Versuchen mit *B. coli*, *B. aerogenes* und *Staph. aureus*, die in flüssigen Medien[15] bei $p_H$-Werten zwischen 6,2 und 6,5 6 Stunden bei 37° gezüchtet worden waren, ergab sich nun, dass das abzentrifugierte Medium im Verlaufe von einigen Minuten eine sodaalkalische Lösung von 3,4-Dinitrobenzoesäure[14] nicht reduzieren konnte. Nennenswerte Mengen an stark reduzierender Substanz waren also nicht vorhanden.

Die abzentrifugierten Bakterien aber gaben im Verlaufe von wenigen Minuten in ein wenig Nährmedium suspendiert nach Zusatz von 0,2 ccm einer 1-proz. Lösung von 3,4-Dinitrobenzoesäure in 2-n Sodalösung eine starke Violettfärbung ($p_H$-Wert alsdann 10,2 bis 10,4). Die Reduktionswirkung solcher Bakteriensuspensionen gegenüber sodaalkalischen o-Dinitrobenzollösungen nach O'Meara kann also bestätigt werden.

Versuche, die Reduktionswirkung der Bakterien auf Redukton zurückzuführen, schlugen jedoch fehl. So zerstörte kurzes Aufkochen der Bakterien in Gegenwart von ein wenig Nährmedium oder dest. Wasser die Reduktionskraft. Eine einer solchen Bakteriensuspension zugesetzte Menge an Redukton, die eben zum Nachweis mit dem Dinitrobenzoesäurereagens ausreichte, blieb bei solchen Erhitzungsversuchen erhalten. Es fehlt somit bis jetzt jeder direkte Nachweis für die Bildung von Redukton durch die Bakterien. Die von O'Meara angegebene reduzierende Wirkung von Bakterien gegenüber o-Dinitrobenzol, die von uns mit Dinitrobenzoesäure bestätigt werden konnte, kann auch durch eine enzymatische Reduktion zustande kommen. Somit entbehren auch die Spekulationen über die Hemmwirkung der Sulfonamide (: Abfangen von Redukton) einer gesicherten Grundlage.

Es ist beabsichtigt, die Versuche zum direkten papierchromatographischen Nachweis einer eventuellen Reduktonbildung durch Bakterien an einem grösseren Versuchsmaterial fortzusetzen.

Herrn Prof. Hans von Euler und dem Svenska Institutet danke ich herzlichst für die Einladung nach Schweden, die die Durchführung dieser Arbeit ermöglichte. Hrn. Ing. Hans Hasselquist und Hrn. Armin Glaser danke ich für die freundliche Unterstützung bei der Durchführung der Versuche.

Stockholm, Universität, Inst. f. organ.-chem. Forschung und Vitamininstitut.

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[15] Glukose-Bouillon.

Tryckt 8 december 1950

Uppsala 1950. Almqvist & Wiksells Boktryckeri AB

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# The crystal structure of $KSb_4F_{13}$ and isomorphous compounds

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With 2 figures in the text

In a previous investigation at this Institute (2) it was shown that the struc-ure of antimony trifluoride is probably built up of $SbF_3$ molecules, the three Sb–F distances being 2.0 Å. Earlier investigations by RAD and HAUSER (6), by FLÜCKIGER (5), by EPHRAIM and BARTECZKO (4), and by WELLS and METZGER (7) showed that antimony trifluoride forms compounds with alkalifluorides including $NH_4F$ and TlF) in the proportions 4 : 1, 3 : 1, 2 : 1, 1 : 1, 1 : 2, and 1 : 3.[1] We have verified this by extensive X-ray work on all these compounds except the 1 : 3 one. (According to FLÜCKIGER this compound exists in the system $SbF_3$-NaF, and the Na compounds have not been investigated so far.)

Obviously, in all these compounds between $SbF_3$ and alkalifluorides, the ratio F : Sb exceeds 3 : 1. Thus, in their structures one of the following arrangements must occur:

1) The structure is built up of $SbF_3$ groups, $F^-$ ions and alkali ions, with no bonds between the $F^-$ ions and the antimony atoms, the $F^-$ ions forming bonds with the alkali ions only.

2) The antimony atoms form a larger number of bonds than in the antimony trifluoride, the bonds being all of the same length. It seems that there are probably three *p* bonds in antimony fluoride. This mechanism for the formation of the complex compounds necessitates the antimony bonds being of another type.

3) The three short Sb–F bonds are also found in the complex compounds, but that in addition to these bonds the antimony atoms form 1–3 (or more) secondary bonds with significantly longer Sb–F distances.

As will be seen the structure determinations for the $RSb_4F_{13}$ compounds ndicate that the third possibility is valid in their case.

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[1] According to WELLS and METZGER a compound with the formula $Cs_4Sb_7F_{25}$ also exists. We have not been able to verify this.

## Preparation of the compounds

RAD and HAUSER (6) showed that by adding $(NH_4)_2HPO_4$ to a solution of $Sb_2O_3$ in HF in the ratio $NH_4 : Sb = 1 : 4$ a compound of composition $NH_4Sb_4F_{13}$ was obtained in the form of prismatic, transparent needles. The formula was confirmed by BARTECZKO who made a complete analysis of the phase.

In our syntheses of the $RSb_4F_{13}$ phases, the alkali carbonate, either $RHCO_3$ or $R_2CO_3$, and $Sb_2O_3$ in the proportions $R : Sb\ \ 1 : 12$ was dissolved in an excess of dilute hydrofluoric acid. The solution was evaporated just to dryness, the solid residue immediately dissolved in water and left to crystallize at room temperature. The first fraction consisted of the $RSb_4F_{13}$ phase. The habit of the crystals varied from rather elongated prismatic needles ($NH_4$, K) to short prisms (Cs).

## Unit cell and space group of the $RSb_4F_{13}$ compounds

The X-ray investigation showed that the $RSb_4F_{13}$ compounds form an isomorphous series with the following cell dimensions (Cr–*K* radiation $\lambda_\alpha = 2.2909$ Å):

| | *a* | *c* |
|---|---|---|
| $KSb_4F_{13}$ | 9.636 ± 0.005 Å | 6.365 ± 0.003 Å |
| $RbSb_4F_{13}$ | 9.635 | 6.563 |
| $CsSb_4F_{13}$ | 9.639 | 6.785 |
| $NH_4Sb_4F_{13}$ | 9.643 | 6.553 |
| $TlSb_4F_{13}$ | 9.637 | 6.584 |

The Laue and Weissenberg photographs showed that the space group was $C_{4h}^5 - I\,4/m$, $C_4^5 - I\,4$, or $S_4^2 - I\,\bar{4}$.

The density of the Tl-compound was found to be 5.01, which corresponds to two groups of $TlSb_4F_{13}$ in the unit cell ($d_{\text{theor.}} = 5.13$).

## The positions of the metal atoms

In all the possible space groups the two R atoms can be placed arbitrarily at $0\,0\,\frac{1}{2}$; $\frac{1}{2}\,\frac{1}{2}\,0$. A rough comparison between observed and calculated intensities showed that the eight antimony atoms can only be placed in one way: in an eigthfold position $x\,y\,0$ with $2\pi x$ 105–110° and $2\pi y$ 5–10°. As the sign of the structure factors of almost all the reflexions could now be safely established, two-dimensional Fourier-summations in the $xy$-plane were made for the K, $NH_4$, Cs, and Tl-compounds.

The signs of the structure factors can in fact be deduced for almost all reflexions $h\,k\,0$ without a preliminary trial and error process from the assumptions that the R atoms are situated at $0\,0\,\frac{1}{2}$; $\frac{1}{2}\,\frac{1}{2}\,0$ and that the Sb and F parameters are approximately the same in the compounds. The relative intensities of the reflexions of the K (or $NH_4$), Cs, and Tl compounds are compared. If for a reflexion $h k 0$ $I(K) < I(Cs) < I(Tl)$, then $F(h k 0)$ is positive; if $I(K) > I(Cs) > I(Tl)$ then $F(h k 0)$ is negative, and if $I(Cs) = 0$ and $I(Tl) \sim I(K)$, then $F(h k 0)$ is positive for the Tl compound but negative for the K compound.

The Fourier projections gave the following parameters for the antimony atoms:

| | $2\pi x$ | $2\pi y$ |
|---|---|---|
| $KSb_4F_{13}$ | 107° ± 0.5° | 7° ± 0.5° |
| $NH_4Sb_4F_{13}$ | 107° | 6° |
| $CsSb_4F_{13}$ | 108° | 6° |
| $TlSb_4F_{13}$ | 108° | 6° |

## The positions of the fluorine atoms

In addition to the maxima from the R atoms and the antimony atoms some small peaks appeared at the following positions (Fig. 1):

| $KSb_4F_{13}$ | | | $NH_4Sb_4F_{13}$ | | | $TlSb_4F_{13}$ | | | $CsSb_4F_{13}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $2\pi x$ | $2\pi y$ | h | $2\pi x$ | $2\pi y$ | h | $2\pi x$ | $2\pi y$ | h | $2\pi x$ | $2\pi y$ | h |
| 0 | 180 | 170 | 0 | 180 | 120 | 0 | 180 | 120 | 0 | 180 | 130 |
| 15 | 60 | 120 | — | — | — | — | — | — | — | — | — |
| 30 | 85 | 180 | 30 | 85 | 130 | 30 | 80 | 90 | 30 | 60 | 130 |
| — | — | — | — | — | — | 40 | 20 | 90 | — | — | — |
| — | — | — | — | — | — | — | — | — | 70 | 6 | 180 |
| 135 | 35 | 200 | 130 | 35 | 200 | 135 | 30 | 130 | 125 | 45 | 110 |
| 160 | 40 | 150 | 165 | 45 | 120 | 160 | 50 | 60 | 150 | 75 | 130 |

As will be seen, a maximum appears in all cases for $2\pi x = 0$, $2\pi y = 180°$. As there are 26 F-atoms in the unit cell and as the symmetry only allows of 2, 4, 8, and 16-fold positions, at least one two-fold position must be occupied by F-atoms. In the relevant space groups, the two-fold positions have the $xy$-coordinates $0\,0$; $\frac{1}{2}\,\frac{1}{2}$ ($C_{4h}^5$, $C_4^5$, and $S_4^2$) or $0\,\frac{1}{2}$; $\frac{1}{2}\,0$ ($S_4^2$). The Fourier projections indicate that the F-atoms occupy the latter positions, giving the space group $S_4^2$. However, as the conclusions from the Fourier projections concerning the arrangement of the F-atoms may be unreliable, both these two-fold positions will be discussed.

### A. The space group $S_4^2$

As a consideration of the available space shows that the 4-fold positions in $S_4^2$ cannot possibly be occupied by fluorine atoms, it follows that the 24 remaining F-atoms must be distributed over three 8-fold positions. It seems that these

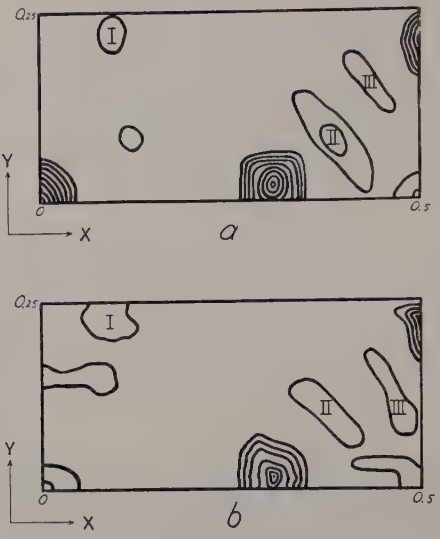

Fig. 1. *a* $TlSb_4F_{13}$ Projection of the electron density on (0 0 1). *b* $KSb_4F_{13}$ Projection of the electron density on (0 0 1).

positions probably correspond to all or some of the peaks in the Fourier-summations common to all the four compounds (see the above table). These three peaks have the coordinates (mean values).

| | $2\pi x$ | $2\pi y$ |
|---|---|---|
| I ......................... | 30° | 80° |
| II ......................... | 130° | 35° |
| III ......................... | 160° | 50° |

A consideration of the interatomic distances shows that either position II or III, but not both, can be occupied by fluorine atoms. As the peak corresponding to II is the higher one in three out of four summations (see the table p. 19), position II is more probable than position III.

A comparison of the observed $F$-values with the structure factors of the metal atoms for the K and $NH_4$ compounds shows that conclusions can be drawn in some cases concerning the sign and magnitude of the sum of the structure factors of the fluorine atoms. Only reflexions with rather low indices were considered, so that errors arising from inaccuracies in the parameter determinations for the antimony atoms would be of little or no importance.

The estimation of the absorption effects presents some difficulties, but a careful comparison with adjoining reflexions shows that the following signs and magnitudes might be expected for the sum of the structure factors of the fluorine atoms:

| $h\,k\,l$ | Sign | Magnitude | $F$calc. for F-atoms | |
|---|---|---|---|---|
| | | | $S_4^2$ | $C_{4h}^5$ (see p. 24) |
| 310 | — | medium to large | —87 | —57 |
| 130 | + | small to medium | 36 | 66 |
| 420 | — | small | —33 | —33 |
| 240 | + | large | 140 | 140 |
| 640 | + or — | small | —8 | —8 |
| 550 | — | medium to large | —64 | —48 |
| 460 | + or — | small | —3 | —3 |
| 930 | — | medium | —50 | —40 |
| 390 | + | small to medium | 20 | 30 |

These criteria for the structure factors of the fluorine atoms, combined with considerations of the available space and with the deductions from the Fourier-syntheses, lead to the following atomic positions and parameters for this alternative:

Space group $S_4^2 - I\,\bar{4}$ (parameters for $KSb_4F_{13}$)

$(0\,0\,0;\ \frac{1}{2}\,\frac{1}{2}\,\frac{1}{2}) +$

2 R in 2 $(b)$: $0\,0\,\frac{1}{2}$

8 Sb in 8 $(g)$: $x\,y\,z;\ \bar{x}\,\bar{y}\,z;\ y\,\bar{x}\,\bar{z};\ \bar{y}\,x\,\bar{z}$ $x_1 = 0.298$ $y_1 = 0.0194$ $z_1 = 0.000$

2 $F_1$ in 2 $(c)$: $0\,\frac{1}{2}\,\frac{1}{4}$[1]

8 $F_2$ in 8 $(g)$: $x_2\,y_2\,z_2$ $x_2 = -0.153$ $y_2 = -0.403$ $z_2 = 0.00$

8 $F_3$ in 8 $(g)$: $x_3\,y_3\,z_3$ $x_3 = 0.097$ $y_3 = 0.208$ $z_3 = 0.236$

8 $F_3$ in 8 $(g)$: $x_4\,y_4\,z_4$ $x_4 = 0.097$ $y_4 = 0.208$ $z_4 = -0.236$

The agreement between observed and calculated $F$-values is satisfactory but not complete (Table 1). Extensive trial and error calculations have shown no possibility for eliminating the few discrepancies which still exist. In addition to still existing errors in the parameters, these difficulties may depend on a non-spherical distribution of the electrons around the atoms. This effect may be of significance particularly with the antimony atoms.

## B. Space group $C_{4h}^5$

Two fluorine atoms are placed at 2 $(a)$: $0\,0\,0;\ \frac{1}{2}\,\frac{1}{2}\,\frac{1}{2}$. For the other atoms considerations of the intensities and the available space lead to the same arrangement as in $S_4^2$ with the atoms in the following positions:

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[1] See discussion of the alternative arrangement.

*Table 1*

$KSb_4F_{13}$. Comparison between observed and calculated $F$-values. Cu–$K$. The column $F_{calc}\ S_4^2$ shows the intensities calculated for alternative A (see p. 21) the column $F_{calc}\ C_{4h}^5$ those for alternative B (see p. 24).

| $h\,k\,l$ | $F_{obs}$ | $F_{calc}$ | |
|---|---|---|---|
| | | $S_4^2$ | $C_{4h}^5$ |
| 200 | 50 | 39 | 39 |
| 400 | 130 | 184 | 184 |
| 600 | 90 | 128 | 128 |
| 800 | <40 | 2 | 2 |
| 1000 | 145 | 192 | 192 |
| 1200 | 80 | −55 | −55 |
| 110 | 50 | −103 | 75 |
| 310 | 80 | 100 | 130 |
| 220 | 190 | −282 | −282 |
| 130 | 45 | 79 | 109 |
| 510 | 55 | −40 | −18 |
| 420 | 110 | −142 | −142 |
| 330 | 220 | 247 | 273 |
| 240 | 95 | 185 | 185 |
| 150 | 150 | −228 | −206 |
| 710 | 140 | 166 | 180 |
| 620 | 30 | 38 | 38 |
| 530 | 85 | −51 | −32 |
| 440 | 120 | 117 | 117 |
| 350 | <40 | −5 | 13 |
| 260 | 30 | −12 | −12 |
| 170 | 40 | −37 | −23 |
| 910 | 50 | 65 | 77 |
| 820 | 160 | −176 | −176 |
| 730 | 90 | 85 | 99 |
| 640 | 230 | 201 | 201 |
| 550 | 250 | −248 | −232 |
| 460 | 40 | −62 | −62 |
| 370 | 290 | 281 | 295 |
| 280 | 65 | −60 | −60 |
| 190 | 160 | −134 | −122 |
| 1110 | 55 | 79 | 89 |
| 1020 | 60 | 32 | 32 |
| 930 | 40 | −67 | −57 |
| 840 | <50 | −2 | −2 |
| 750 | <50 | 34 | 46 |
| 660 | 57 | 40 | 40 |
| 570 | <50 | 49 | 61 |
| 480 | 90 | −30 | −30 |
| 390 | 45 | 54 | 64 |
| 2 10 0 | 115 | 109 | 109 |
| 1 11 0 | 110 | −97 | −87 |
| 1220 | 190 | −150 | −150 |
| 1130 | 95 | −44 | −34 |
| 1040 | 240 | 209 | 209 |
| 950 | <50 | 6 | 16 |
| 860 | 200 | −126 | −126 |
| 770 | 135 | 147 | 157 |
| 680 | 105 | 97 | 97 |
| 590 | 115 | −156 | −146 |
| 4 10 0 | <50 | 19 | 19 |
| 3 11 0 | 115 | 69 | 79 |
| 2 12 0 | 40 | −18 | −18 |
| 1150 | 75 | −50 | −40 |
| 1060 | <40 | 20 | 20 |
| 970 | 125 | 113 | 123 |
| 880 | 95 | −60 | −60 |
| 790 | 115 | −134 | −124 |
| 6 10 0 | 165 | 174 | 174 |
| 5 11 0 | 60 | 44 | 54 |
| 981 | 45 | 43 | 51 |
| 871 | 45 | 11 | 1 |
| 761 | 75 | 44 | −34 |
| 651 | 40 | 10 | −2 |
| 541 | 75 | 57 | −48 |
| 431 | 30 | 58 | 67 |
| 321 | 55 | 27 | −13 |
| 211 | 135 | 201 | −188 |
| 101 | 115 | 100 | 116 |
| 121 | 135 | 287 | −273 |
| 231 | 65 | 90 | −76 |
| 341 | 220 | 222 | 232 |
| 451 | 180 | 161 | −153 |
| 561 | 210 | 140 | −136 |
| 671 | 240 | 173 | 178 |
| 781 | <45 | 15 | −10 |
| 891 | 150 | 182 | −178 |
| 1071 | 95 | 124 | 129 |
| 961 | 60 | 64 | −59 |
| 851 | 240 | 179 | −174 |
| 741 | 210 | 148 | 154 |
| 631 | 135 | 99 | 107 |
| 521 | 230 | 318 | −308 |
| 411 | 55 | 44 | 54 |
| 301 | 170 | 237 | 252 |
| 141 | 55 | 47 | −34 |
| 251 | 140 | 190 | −180 |

Table 1 (cont.).

| $h\,k\,l$ | $F_{obs}$ | $F_{calc}$ |  | $h\,k\,l$ | $F_{obs}$ | $F_{calc}$ |  |
|---|---|---|---|---|---|---|---|
|  |  | $S_4^2$ | $C_{4h}^5$ |  |  | $S_4^2$ | $C_{4h}^5$ |
| 361 | 135 | 95 | 103 | 352 | 45 | 2 | 2 |
| 471 | 55 | 25 | 30 | 262 | <45 | 0 | 16 |
| 581 | 95 | 89 | −84 | 172 | 40 | 90 | 90 |
| 691 | <50 | 16 | −10 |  |  |  |  |
| 7 10 1 | <50 | 23 | 27 | 912 | 45 | 32 | 32 |
| 1051 | 50 | 37 | 31 | 822 | 180 | 150 | −138 |
| 941 | 165 | 106 | 111 | 732 | 105 | 87 | 87 |
| 831 | 170 | 142 | −136 | 642 | 235 | 189 | 203 |
| 721 | 40 | 48 | −41 | 552 | 210 | 114 | −114 |
| 611 | 65 | 74 | 82 | 462 | 85 | 36 | −22 |
| 501 | 45 | 29 | −17 | 372 | 260 | 235 | 235 |
| 161 | 120 | 159 | −150 | 282 | 65 | 68 | −56 |
| 271 | 105 | 90 | 97 | 192 | 115 | 142 | −142 |
| 381 | 65 | 54 | 60 |  |  |  |  |
| 491 | 185 | 161 | −156 | 1112 | 115 | 81 | 81 |
| 5 10 1 | 50 | 7 | 10 | 1022 | 60 | 21 | 31 |
|  |  |  |  | 932 | <55 | 21 | 21 |
| 1031 | 65 | 41 | 46 | 842 | <55 | 29 | 39 |
| 921 | 150 | 133 | −127 | 752 | <55 | 7 | 7 |
| 811 | 40 | 35 | −28 | 662 | 55 | 26 | 38 |
| 701 | 155 | 168 | 195 | 572 | 80 | 46 | 46 |
| 181 | 190 | 196 | −190 | 482 | 110 | 120 | −110 |
| 291 | 75 | 73 | −67 | 392 | 65 | 41 | 41 |
| 3 10 1 | 150 | 144 | 149 | 2 10 2 | 165 | 138 | 148 |
|  |  |  |  | 1 11 2 | 115 | 81 | −81 |
| 1121 | 125 | 125 | −120 | 1132 | 90 | 61 | −61 |
| 1011 | 200 | 177 | 182 | 1042 | 195 | 194 | 204 |
| 901 | <50 | 14 | −7 | 952 | <55 | 7 | −7 |
| 1 10 1 | <50 | 22 | −16 | 862 | 200 | 206 | −196 |
| 2 11 1 | 50 | 29 | 34 | 772 | 160 | 126 | 126 |
|  |  |  |  | 682 | 160 | 125 | 135 |
| 202 | 40 | 94 | 94 | 592 | 160 | 137 | −137 |
| 402 | 150 | 199 | 199 | 4 10 2 | <55 | 22 | 32 |
| 602 | 105 | 177 | 177 | 3 11 2 | 130 | 140 | 140 |
| 802 | <45 | 16 | 16 |  |  |  |  |
| 1007 | 170 | 115 | 115 | 1062 | <45 | 37 | 45 |
| 1202 | 125 | 38 | −38 | 972 | 150 | 126 | 126 |
|  |  |  |  | 882 | 90 | 62 | −52 |
| 112 | 35 | 109 | 109 | 792 | 105 | 53 | −53 |
|  |  |  |  | 6 10 2 | 155 | 182 | 190 |
| 312 | 240 | 300 | 300 |  |  |  |  |
| 222 | 220 | 222 | −202 | 002 | 225 | 346 | 346 |
| 132 | 80 | 24 | 24 | 004 | 250 | 406 | 406 |
| 512 | 40 | 64 | −64 | 006 | 190 | 224 | 224 |
| 422 | 85 | 136 | −116 | 008 | 215 | 258 | 258 |
| 332 | 285 | 222 | 222 | 103 | 70 | 64 | 78 |
| 242 | <35 | 26 | −4 | 303 | 170 | 226 | 236 |
| 152 | 180 | 233 | −233 | 503 | 70 | 25 | −16 |
|  |  |  |  | 703 | 230 | 186 | 192 |
| 712 | 120 | 161 | 161 | 903 | <90 | 23 | −14 |
| 622 | 70 | 122 | −106 | 1103 | <70 | 7 | 0 |
| 532 | 90 | 89 | −89 |  |  |  |  |
| 442 | 145 | 148 | 164 | 204 | <70 | 29 | 29 |

Table 1 (cont.).

| $h\,k\,l$ | $F_{obs}$ | $F_{calc}$ $S_4^2$ | $F_{calc}$ $C_{4h}^5$ | $h\,k\,l$ | $F_{obs}$ | $F_{calc}$ $S_4^2$ | $F_{calc}$ $C_{4h}^5$ |
|---|---|---|---|---|---|---|---|
| 404 | 145 | 152 | 152 | 905 | $<65$ | 5 | 4 |
| 604 | 80 | 107 | 107 | 206 | 65 | 48 | 48 |
| 804 | $<90$ | 0 | 0 | 406 | 130 | 141 | 141 |
| 1004 | 250 | 176 | 176 | 606 | 115 | 126 | 126 |
| | | | | 806 | $<55$ | 13 | 13 |
| 105 | 80 | 75 | 62 | | | | |
| 305 | 155 | 166 | 172 | 107 | $<80$ | 39 | 44 |
| 505 | $<80$ | 20 | $-13$ | 307 | 140 | 169 | 159 |
| 705 | 150 | 131 | 136 | 507 | $<70$ | 21 | $-15$ |

$C_{4h}^5 - I\,4/m\ (0\,0\,0;\ \tfrac{1}{2}\,\tfrac{1}{2}\,\tfrac{1}{2}) +$

2 R in 2 $(b)$: $0\,0\,\tfrac{1}{2}$

8 Sb in 8 $(h)$: $\pm(x\,y\,0);\ \pm(y\,\bar{x}\,0)$. $x = 0.298$ $y = 0.0194$

2 $F_1$ in 2 $(a)$: $0\,0\,0$

8 $F_2$ in 8 $(h)$: $x\,y\,0$. $x = -0.153$ $y = -0.403$

16 $F_3$ in 16 $(i)$: $\pm(x\,y\,z);\ \pm(\bar{x}\,\bar{y}\,z);\ \pm(\bar{y}\,x\,z);\ \pm(y\,\bar{x}\,z)$.

$x = 0.097$ $y = 0.208$ $z = 0.236$

A comparison between observed and calculated $F$-values with this alternative is given in Table 1.

## Comparison of the two possible arrangements of the fluorine atoms

The two possible arrangements of the fluorine atoms lead to the following interatomic distances:

| A. Arrangement in $S_4^2$ | B. Arrangement in $C_{4h}^5$ |
|---|---|
| Sb–$F_2$ $= 1.94 \pm 0.1$ Å | Sb–$F_2$ $= 1.94 \pm 0.1$ Å |
| Sb–2 $F_3$ $= 2.06 \pm 0.1$ | Sb–2 $F_3$ $= 2.06 \pm 0.1$ |
| 4 Sb–$F_1$ $= 2.53 \pm 0.03$ | 4 Sb–$F_1$ $= 2.87 \pm 0.03$ |
| Sb–2 $F_3$ $= 3.04 \pm 0.1$ | Sb–2 $F_3$ $= 3.04 \pm 0.1$ |
| K–8 $F_3$ $= 2.72 \pm 0.1$ | K–8 $F_3$ $= 2.72 \pm 0.1$ |
| K–4 $F_2$ $= 3.48 \pm 0.1$ | K–4 $F_2$ $= 3.48 \pm 0.1$ |
| | K–2 $F_1$ $= 3.18 \pm 0.01$ |
| $F_1$–$F_2$ $= 2.36 \pm 0.1$ | $F_1$–$F_2$ $= 2.66 \pm 0.1$ |
| $F_1$–$F_3$ $= 2.96 \pm 0.1$ | $F_1$–$F_3$ $= 2.96 \pm 0.1$ |
| $F_2$–$F_3$ $= 2.46 \pm 0.2$ | $F_2$–$F_3$ $= 2.46 \pm 0.2$ |

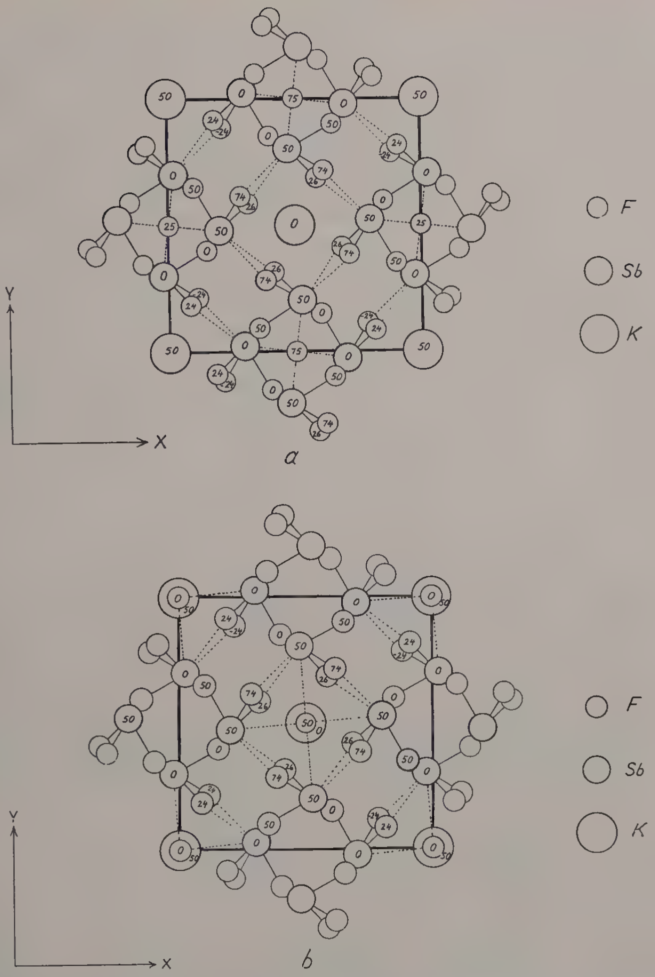

Fig. 2 *a* shows the positions of the atoms in $S_4^2$ (alternative A in the discussion) and Fig. 2 *b* the atoms in $C_{4h}^5$ (alternative B). Superimposed oxygen atoms are symetrically displaced. The bonds within the $SbF_3$ molecules are shown with fulldrawn lines, and the longer (secondary) Sb—F bonds with dotted lines.

As will be seen, some very short F—F distances of only 2.36 ± 0.1 Å are obtained with arrangement A. It is also difficult to explain the variation of the *c*-axis with the size of the alkali ion in this alternative. As the *a*-axis is practically the same for all the compounds in this isomorphous series, it is probable that all the *x* and *y* parameters are approximately the same in the different compounds. This has been verified for the antimony parameters (p. 19). As only very small variations in the Sb—F distances can be expected in the different compounds of the isomorphous series, it follows that the *z*-

*Table 2*

$TlSb_4F_{13}$. Comparison between observed and calculated $F$-values. Cu–$K$ radiation. $F_{calc}$ I shows the $F$-values calculated with the Tl and Sb atoms in separate positions, $F_{calc}$ II with these atoms in statistical distribution. The alternatives A and B (see p. 21 and 24) for the fluorine atom arrangement are given in the columns labelled $S_4^2$ and $C_{4h}^5$ respectively.

| $h\,k\,l$ | $F_{obs}$ | $F_{calc}$ I | | $F_{calc}$ II | |
|---|---|---|---|---|---|
| | | $S_4^2$ | $C_{4h}^5$ | $S_4^2$ | $C_{4h}^5$ |
| 200 | 100 | 162 | 162 | 120 | 120 |
| 400 | 160 | 285 | 285 | 265 | 265 |
| 600 | 160 | 246 | 246 | 230 | 230 |
| 800 | 60 | 88 | 88 | 52 | 52 |
| 1000 | 230 | 283 | 283 | 274 | 274 |
| 1200 | <45 | 46 | 46 | 11 | 11 |
| 110 | <40 | 17 | 55 | −38 | 0 |
| 310 | 170 | 202 | 232 | 184 | 214 |
| 220 | 120 | −164 | −164 | −244 | −244 |
| 130 | 120 | 203 | 233 | 166 | 196 |
| 510 | <60 | 45 | 67 | −10 | 12 |
| 420 | 80 | −40 | −40 | −99 | −99 |
| 330 | 280 | 369 | 392 | 358 | 381 |
| 240 | 140 | 271 | 271 | 229 | 229 |
| 150 | 130 | −100 | −78 | −173 | −151 |
| 710 | 290 | 232 | 246 | 217 | 231 |
| 620 | 70 | 137 | 137 | 90 | 90 |
| 530 | 60 | 65 | 83 | 13 | 31 |
| 440 | 230 | 201 | 201 | 173 | 173 |
| 350 | 60 | 83 | 101 | 45 | 63 |
| 260 | 100 | 90 | 90 | 48 | 48 |
| 170 | 50 | 50 | 64 | 14 | 28 |
| 910 | 130 | 142 | 154 | 117 | 129 |
| 820 | 180 | −106 | −106 | −169 | −169 |
| 730 | 220 | 185 | 199 | 173 | 187 |
| 640 | 380 | 282 | 282 | 273 | 273 |
| 550 | 240 | −171 | −155 | −239 | −223 |
| 460 | 60 | 53 | 53 | 10 | 10 |
| 370 | 300 | 362 | 376 | 363 | 377 |
| 280 | <60 | −6 | −6 | −50 | −50 |
| 190 | 130 | −35 | −23 | −88 | −76 |
| 1110 | 65 | 126 | 136 | 96 | 106 |
| 1020 | 120 | 88 | 88 | 60 | 60 |
| 930 | <60 | 40 | 50 | 10 | 20 |
| 840 | 60 | 67 | 67 | 34 | 34 |
| 750 | 60 | 93 | 105 | 55 | 67 |
| 660 | 190 | 141 | 141 | 116 | 116 |
| 570 | 70 | 124 | 136 | 98 | 110 |
| 480 | 60 | 34 | 34 | −14 | −14 |
| 390 | 100 | 149 | 159 | 124 | 134 |

Table 2 (cont.).

| $h\,k\,l$ | $F_{\text{obs}}$ | $F_{\text{calc}}$ I |  | $F_{\text{calc}}$ II |  |
|---|---|---|---|---|---|
|  |  | $S_4^2$ | $C_{4h}^5$ | $S_4^2$ | $C_{4h}^5$ |
| 2 10 0 | 135 | 174 | 174 | 159 | 159 |
| 1 11 0 | 95 | −45 | −35 | −94 | −84 |
| 1220 | 120 | −89 | −89 | −139 | −139 |
| 1130 | 80 | 38 | 48 | −6 | 4 |
| 1040 | 350 | 269 | 269 | 269 | 269 |
| 950 | 70 | 45 | 55 | 10 | 20 |
| 860 | 215 | −33 | −33 | −84 | −84 |
| 770 | 250 | 226 | 236 | 213 | 223 |
| 680 | 175 | 145 | 145 | 123 | 123 |
| 590 | 150 | −68 | −58 | −120 | −110 |
| 4 10 0 | 100 | 108 | 108 | 82 | 82 |
| 3 11 0 | 80 | 120 | 130 | 103 | 113 |
| 2 12 0 | <50 | 40 | 40 | 4 | 4 |
| 1150 | 120 | −14 | −4 | −57 | −47 |
| 1060 | 85 | 101 | 101 | 75 | 75 |
| 970 | 240 | 194 | 205 | 183 | 193 |
| 880 | 115 | −18 | −18 | −61 | −61 |
| 790 | 95 | −51 | −41 | −92 | −82 |
| 6 10 0 | 300 | 255 | 255 | 251 | 251 |
| 5 11 0 | 50 | 79 | 89 | 47 | 57 |

arameters of the fluorine atoms must also be approximately the same in all he compounds. With alternative A, the K–F and Cs–F distances are:

$$\text{K–8 F}_3 = 2.67\ \text{Å} \qquad \text{Cs–8 F}_3 = 2.79\ \text{Å}$$
$$\text{K–4 F}_2 = 3.48 \qquad \text{Cs–4 F}_2 = 3.48$$

As the ionic radius of $K^+$ is 1.33 Å and that of $Cs^+$ 1.69 Å, this small ifference in the R–8 F distances is difficult to explain.

With alternative B, these very short F–F distances are not obtained. The orresponding R–F distances are:

$$\text{K–2 F}_1 = 3.18 \qquad \text{Cs–2 F}_1 = 3.39$$
$$\text{K–8 F}_3 = 2.67 \qquad \text{Cs–8 F}_3 = 2.79$$
$$\text{K–4 F}_2 = 3.48 \qquad \text{Cs–4 F}_2 = 3.48$$

The small variation in the R–8 F distances can in this case be explained n the following way. The F atoms situated above and below the R atom see Fig. 2 *b*) polarize the R atom. As the polarizibility is larger for the Cs tom than for the K atom, the Cs atoms will be more deformed (elongated in he *c*-direction) than the K atoms and an increase in the R–8 F distances vhich is less than the difference in ionic size is to be expected.

A study of the configuration around the antimony atoms shows that in case A these atoms are surrounded by three F atoms at 2.0 Å (mean value), one F atom at 2.5 Å, and two F atoms at 3.0 Å. These six F atoms form a distorted trigonal prism around the Sb atom. In case B, there are three F atoms at 2.0 Å, one F atom at 2.9, and two F atoms at 3.0 Å. The six F atoms form a very distorted octahedron around the Sb atom. The longer (secondary) Sb–F bonds bind the $SbF_3$ molecules together to infinite, two-dimensional complexes $(Sb_4F_{13})^-_\infty$ in alternative A, and to finite $Sb_4F_{13}$ groups in alternative B. As distorted octahedral configurations are also found for the Sb atoms in the cubic modification of $Sb_2O_3$ with corresponding interatomic distances (Sb–3 O = 2.0 Å; Sb–3 O = 2.9 Å, see Almin and Westgren (1)), this fact also appears to argue in favour of alternative B.

Thus the only reason in favour of alternative A is that maxima corresponding to the positions of the two F atoms in $0\,\frac{1}{2}\,\frac{1}{4}$; $\frac{1}{2}\,0\,\frac{3}{4}$ are found in the Fourier projections. However, this argument is weakened by the fact that deductions of the positions of the fluorine atoms from the Fourier projections are rather uncertain in the case of compounds of this type because of the very unfavourable relation between the scattering powers of the fluorine atoms and the antimony atoms. As was mentioned above, there are also a few discrepancies between observed and calculated *F*-values which it has not been possible to eliminate in spite of elaborate trial and error calculations. If these discrepancies were due to errors in the parameters of the fluorine atoms only (the error in the parameters of the Sb atoms is very small, see p. 19), they should be much less for the Tl compound than for the K compound. This is in fact not the case as may be seen by comparing Table 1 and Table 2 and from the discussion in the following section on $TlSb_4F_{13}$.

To sum up, comparison of the dimensions of the unit cells of the compounds $RSb_4F_{13}$ and considerations of the configurations around the antimony atoms indicate that the holohedral arrangement of the fluorine atoms (alternative B) is more likely than the hemihedral arrangement (alternative A) and that these reasons must be considered of greater importance than the evidence from the Fourier analyses.

## $TlSb_4F_{13}$

The Fourier-syntheses of the $xy$-projection showed that the maxima attributed to the thallium and the antimony atoms were of equal height in spite of the large difference in atomic number of the two elements. A careful inspection of the $hk0$ intensities revealed some discrepancies, which were considerably larger than those discussed above for the other compounds in the isomorphous series. As will be seen from Table 2 the agreement between observed and calculated *F*-values is improved in some cases (see the reflexions 590, 860, 880, 1150 in Table 2) if it is assumed that the antimony and thallium atoms are distributed statistically over the ten atomic sites, as may be inferred from the Fourier syntheses. Another possible explanation of the height of the Tl maxima might be that there are about 40 % vacancies among the Tl atoms. This explanation would imply that there are vacancies among the F atoms also, or that there are $H_2O$ molecules among the F atoms, or that some of the Tl atoms are replaced by $H_3O^+$ ions. All these explanations, however, require the

ensity to be considerably lower than that expected for the ideal structure,
e decrease amounting to about 10 %. The observed density is only about
% lower than the calculated one, so that a statistical distribution of the
l and Sb atoms agrees better with the experimental data. However, the dif-
rence in atomic size between $Tl^{+}$ and $Sb^{3+}$ is so considerable that this ex-
lanation does not seem feasible. It must also be pointed out that the length
f the *c*-axis indicates that an atom of about the same size as an Rb-atom
situated in the parameter-free metal position, and this fact rather indicates
compound without solid solution between the Tl and Sb atoms.

Thus it appears that the discrepancies between some of the observed and
e calculated *F*-values in all the compounds $RSb_4F_{13}$ (most marked in the
ompounds with heavy R atoms) can only in part be due to errors in the
arameters. Calculations indicate that the decrements in scattering factors which
ccur, especially when — for the heavy elements — the K absorption edge
es at a shorter wave length than that of the X-ray radiation, can at least
nly partly be responsible for these discrepancies. It seems that some other
isturbance in the scattering factors must occur, maybe caused by a distortion
f the spherical symmetry of the atoms.

We are much indebted to Professor A. WESTGREN for his encouraging in-
erest in this investigation.

This work has been supported by the Swedish Natural Science Research
ouncil.

## SUMMARY

The crystal structures of the compounds $KSb_4F_{13}$, $RbSb_4F_{13}$, $CsSb_4F_{13}$,
$H_4Sb_4F_{13}$, and $TlSb_4F_{13}$ have been determined. The compounds form an iso-
orphous series with tetragonal symmetry. The cell dimensions are:

| | *a* | *c* |
|---|---|---|
| $KSb_4F_{13}$ | 9.636 ± 0.005 Å | 6.365 ± 0.003 Å |
| $RbSb_4F_{13}$ | 9.635 | 6.563 |
| $CsSb_4F_{13}$ | 9.639 | 6.785 |
| $NH_4Sb_4F_{13}$ | 9.643 | 6.553 |
| $TlSb_4F_{13}$ | 9.637 | 6.584 |

The metal atoms are in the following positions:

Space group $C_{4h}^{5} - I\,4/m$

$(0\,0\,0;\ \frac{1}{2}\,\frac{1}{2}\,\frac{1}{2})\ +$

2 K (or Rb, Cs, $NH_4Tl$) in 2 (*b*): $0\,0\,\frac{1}{2}$

8 Sb in 8 (*h*): $x_1\,y_1\,0;\ \bar{x}_1\,\bar{y}_1\,0;\ y_1\,\bar{x}_1\,0;\ \bar{y}_1\,x_1\,0$

$x_1 = 0.298 \quad y_1 = 0.0194$

For the fluorine atoms, the following arrangement is suggested:

$$C_{4h}^{5} - I\,4/m$$

$(0\,0\,0;\ \tfrac{1}{2}\,\tfrac{1}{2}\,\tfrac{1}{2}) +$

2 F in $2(a)$: $0\,0\,0$

8 F in $8(h)$: $x\,y\,0$ $\quad x = -0.153;\ y = -0.403$

16 F in $16(i)$: $\pm(x\,y\,z);\ \pm(\bar{x}\,\bar{y}\,z);\ \pm(\bar{y}\,x\,z);\ \pm(y\,\bar{x}\,z)$

$x = 0.097;\ y = 0.208;\ z = 0.236$

The parameter values given above are calculated for the K compound.

The University of Stockholm, October 1950.

REFERENCES. (1) **Almin, K. E.** and **Westgren, A.**, Arkiv Kemi, Miner. *B 15* No 22 (1942). — (2) **Byström, A.** and **Westgren, A.**, Arkiv Kemi, Miner. *B 17* No 2 (1943). — (3) **Byström, A.**, **Wilhelmi, K. A.**, and **Brotzen, O.**, Acta Chem. Scand. *4*, 1125 (1950). — (4) **Ephraim, F.** and **Barteczko, P.**, Z. anorg. allgem. Chem. *61*, 258—64 (1909). — (5) **Flückiger, S. A.**, Lieb Ann *84*, 248 (1871). — (6) **Rad, A.** and **v. Hauser, G.**, D. P. 50281 (1888). — (7) **Wells, H. L.** and **Metzger, F. J.**, Am. Journ. of Science [4] 11, 451—456 (1901).

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# Influence of fat nutrition upon the growth, the fertility and the longevity of rats. IV

By Beth v. Euler and Hans v. Euler

With 3 figures in the text

Our previous investigations on the influence of nutrition upon the growth, the fertility and the longevity of rats have been recorded in three publications[1]. These contain the results of our experimental series A and B. In both series the animals were given the following diet:

| | |
|---|---|
| Casein | 100 g |
| Wheat starch | 250 g |
| Fat mixture, MW or Butter | 58 g |
| Yeast | 200 g |
| Salt mixture | 25 g |
| Orange juice | 10 ml |
| KJ | 50 mg |

Salt mixture

| | |
|---|---|
| NaCl | 400 g |
| $KH_2PO_4$ | 1600 g |
| $CaH_2(PO_4)_2$ | 1200 g |
| $MgSO_4$ | 400 g |
| Ca-Lactate | 400 g |
| $FeCl_3$ | 40 g |
| $CuSO_4$ | 4 mg |

In these series we stated that the nutritional and physiological value of the fat mixtures, designated MW, which correspond to the margarines manufactured in Sweden during our experimental period, was in no way inferior to the value of the highest quality of Swedish butter.

Concerning the condition of our old rats we found that the main cause of death of old rats whether reared on normal diets or on our experimental diets were pneumonia and nephritis together with other kidney damages. Calcareous deposits were observed mainly in the kidneys.

Our investigations cited above were continued in the years 1947–1949. These experiments are reported in the present paper; they will be discussed elsewhere together with the results of our previous investigations.

When we compared in our previous experimental series the growth rate and the fertility of the rats, receiving our normal synthetic diet with the growth rate and fertility of the rats receiving our stock diet without the fat component, it became evident to us, that our normal synthetic diet, sufficient for shorter experimental periods, is not optimal for longer series which cover the life span of several generations.

We therefore used in all experiments reported in the present publication the following diet:

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[1] Beth v. Euler, Hans v. Euler and Inez Rönnestam-Säberg, I, Vol. 22 A, No. 8 (1946); II. Vol. 24 A, No. 15 (1946); III. 24 A, No. 20 (1947).

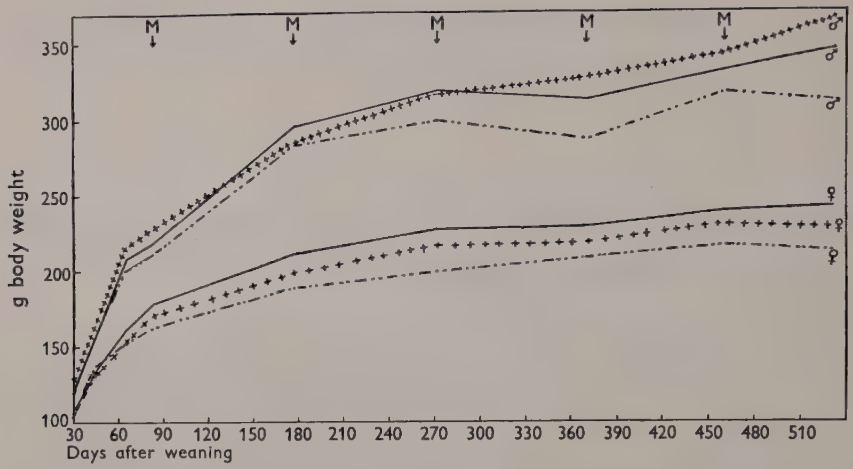

Fig. 1.

———— Butter diet M = Mating
+ + + + Margarine diet
—··—··— Stock diet

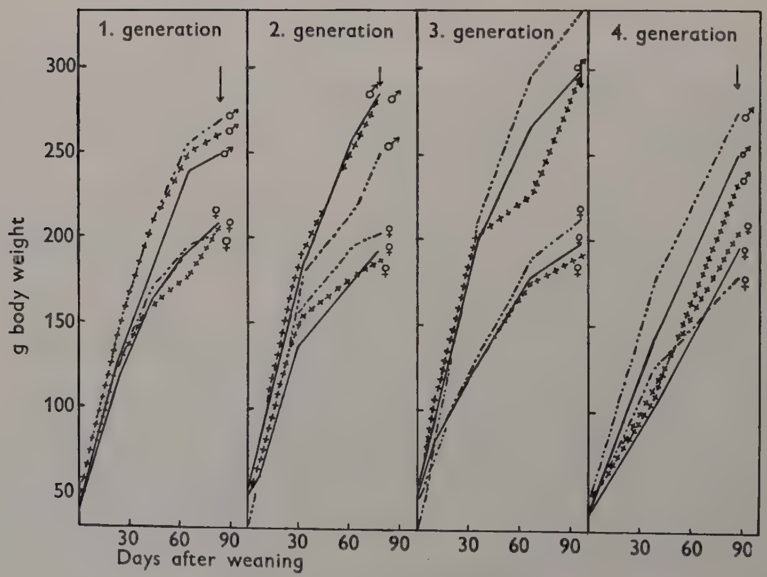

Fig. 2.

———— Butter diet
+ + + + Margarine diet
—··—··— Stock diet

*Table I.*

1. generation mated.

| Diet | Females bred | No. of litters born | | Per cent parturition | Average no. of young per litter | | No. of young of the 2. generation | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | born | red.[1] | born | red.[1] | |
| *Butter diet.* | | | | | | | | | |
| Animals mated 1. time | 56 | 49 | 207 | 87.5 | 8.7 | 6.9 | 426 | (338) | 1794 (1415) |
| 2. » | 56 | 52 | | 92.5 | 9.7 | 7.2 | 473 | (353) | |
| 3. » | 56 | 45 | | 80.4 | 9.7 | 7.5 | 437 | (336) | |
| 4. » | 52 | 36 | | 69.2 | 7.9 | 6.6 | 283 | (236) | |
| 5. » | 43 | 25 | | 58.1 | 7.0 | 6.1 | 152 | (152) | |
| *Marg. diet.* | | | | | | | | | |
| Animals mated 1. time | 56 | 52 | 190 | 92.9 | 9.9 | 7.7 | 516 | (401) | 1761 (1386) |
| 2. » | 56 | 50 | | 89.2 | 9.9 | 7.5 | 497 | (379) | |
| 3. » | 56 | 41 | | 71.4 | 9.1 | 7.3 | 365 | (291) | |
| 4. » | 52 | 27 | | 51.9 | 8.7 | 7.1 | 234 | (190) | |
| 5. » | 42 | 20 | | 47.6 | 7.5 | 6.3 | 149 | (125) | |
| *Stock diet.* | | | | | | | | | |
| Animals mated 1. time | 28 | 23 | 91 | 82.1 | 8.2 | 7.0 | 188 | (161) | 776 (621) |
| 2. » | 28 | 22 | | 78.5 | 9.8 | 7.4 | 215 | (162) | |
| 3. » | 28 | 22 | | 78.6 | 9.4 | 7.0 | 206 | (153) | |
| 4. » | 27 | 13 | | 48.1 | 7.5 | 6.3 | 97 | (82) | |
| 5. » | 20 | 11 | | 55.0 | 6.4 | 5.8 | 70 | (63) | |

| Basal diet. | |
|---|---|
| Skimmilk powder | 24 % |
| Fat | 10 % |
| Wheat powder | 65 % |
| Sodium chloride | 1 % |

| Additions per animal and week | |
|---|---|
| Spinach | 5 g |
| Meat | 5 g |
| Vitamin A | 100 Intern. Units |
| Vitamin D | 5,8 Intern. Units |

Using this diet H. J. DEUEL jr.[2] had gained a very satisfactory growth rate. Our initial task was to compare the influence of the various fats on the growth and the development of our rats over several generations.

The essential problem of this research work concerns the influence of fat nutrition

1) on the physiological condition of our animals during their whole life span, and
2) on their faculty of reproduction, determined quantitativly by the number of litters, and by the weight, the growth rate and the fertility of the offspring.

Unfortunately our measurements, which were planned to cover 8–10 generations in a period of about 3 years, had to be interrupted after 18 months and cover therefore only 4 generations.

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[1] The litters are reduced to a max. number of 8.

[2] H. J. DEUEL jr., Jl. Nutr. 29, 309; 1945. See also the very valuable review by H. J. DEUEL jr. in "Fortschritte der Chemie organ. Naturstoffe" VI (1950).

## I. Influence of fat nutrition on the growth rate of the first generation

In most part of nutritional investigations — also in the recent period of vitamin research — the influence of foodstuffs was determined by the increase of body weight of growing animals. Although "growth" is, from the biochemical point of view, a very complex phenomenon, the increase of body weight indicates at a certain degree the state of health of the animals studied, and — if the experimental period is not too short — the presence or absence of most — but not of all — essential nutritional components.

Many authors have studied the influence of vitamin A, the typical fat-soluble growth-promoting vitamin. Our knowledge on the simultaneous influence of growth accelerating hormones and vitamins is not yet complete, the simultaneous action of the factors promoting and inhibiting growth especially needing further experimental investigations.

This is especially true concerning the influence of diet acting during a long period of the life span and on many generations, and we shall concentrate our report on a period of life exceeding 12 months and on the second and subsequent generations.

Concerning our first generation we shall confine ourselves to the description of the growth curves obtained in our experiments with both sorts of fat to be compared here, namely butter and margarine (fat mixture MW).

*Table II.*

2. generation at the age of 14 days.

| Diet | No. of litters alive | | No. of young alive | | % of no. born (red.) | Average no. young per litter | Average weight g. | | | Total weight of the offspring |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | of the litters | per individual | | |
| *Butter diet.* | | | | | | | | | | |
| Animals | | | | | | | | | | |
| mated 1. time ... | 45 | 190 | 275 | 1219 | 86.1 | 6.4 | 123.5 | 20 | Average (23.5) | 28 625 |
| 2. » | 47 | | 326 | | | | 171.4 | 25 | | |
| 3. » | 43 | | 307 | | | | 173.5 | 24 | | |
| 4. » | 33 | | 193 | | | | 139.8 | 24 | | |
| 5. » | 22 | | 118 | | | | 133.5 | 25 | | |
| *Marg. diet.* | | | | | | | | | | |
| Animals | | | | | | | | | | |
| mated 1. time ... | 49 | 178 | 328 | 1185 | 85.4 | 6.7 | 131.1 | 20 | Average (22.5) | 26 664 |
| 2. » | 49 | | 332 | | | | 162.0 | 24 | | |
| 3. » | 40 | | 271 | | | | 159.5 | 23 | | |
| 4. » | 23 | | 159 | | | | 156.4 | 23 | | |
| 5. » | 17 | | 95 | | | | 137.1 | 24 | | |
| *Stock diet.* | | | | | | | | | | |
| Animals | | | | | | | | | | |
| mated 1. time ... | 22 | 84 | 142 | 523 | 84.2 | 6.2 | 109.2 | 17 | Average (19.4) | 10 123 |
| 2. » | 20 | | 144 | | | | 124.3 | 17 | | |
| 3. » | 20 | | 118 | | | | 119.2 | 20 | | |
| 4. » | 13 | | 69 | | | | 97.4 | 18 | | |
| 5. » | 9 | | 50 | | | | 131.7 | 24 | | |

2. generation at the age of 30 days.

(Cont.)

| Diet | No. of litters alive | No. of young alive | % of no. born (red.) | Average no. young per litter | Average weight g | | | | Total weight of the offspring g |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | of the litter | per individual ♂ | ♀ | Average | |
| *Butter diet.* | | | | | | | | | |
| Animals mated 1. time | 45 | 264 | 83.8 | 6.3 | 286.3 | 51.4 | 47.0 | 53.4 | 63 349 |
| 2. » | 46 | 318 | | | 396.9 | 58.2 | 56.5 | | |
| 3. » | 43 188 | 366 1186 | | | 366.9 | 55.6 | 48.3 | | |
| 4. » | 32 | 183 | | | 304.6 | 54.2 | 52.5 | | |
| 5. » | 22 | 115 | | | 303.9 | 61.4 | 55.6 | | |
| *Marg. diet.* | | | | | | | | | |
| Animals mated 1. time | 49 | 321 | 84.4 | 6.6 | 323.7 | 50.7 | 48.2 | 52.6 | 61 530 |
| 2. » | 49 | 330 | | | 372.9 | 56.3 | 54.3 | | |
| 3. » | 40 178 | 271 1170 | | | 357.6 | 53.4 | 52.2 | | |
| 4. » | 23 | 157 | | | 345.7 | 50.7 | 50.6 | | |
| 5. » | 17 | 91 | | | 302.5 | 57.4 | 55.4 | | |
| *Stock diet.* | | | | | | | | | |
| Animals mated 1. time | 22 | 140 | 82.1 | 6.2 | 228.6 | 36.1 | 35.8 | 40.8 | 20 815 |
| 2. » | 20 | 144 | | | 294.5 | 40.5 | 41.4 | | |
| 3. » | 20 82 | 118 510 | | | 250.0 | 42.4 | 42.4 | | |
| 4. » | 12 | 64 | | | 223.3 | 43.7 | 40.2 | | |
| 5. » | 6 | 44 | | | 276.8 | 49.7 | 51.0 | | |

2. generation mated.

| Diet | Females bred | No. of litters born | Per cent parturition | Average no. of young per litter born red. | No. of young of the 3. generation born |
|---|---|---|---|---|---|
| Butter diet ............ | 8 | 8 | 100 | 11.1 (8.0) | 89 (64) |
| Marg. diet ............. | 8 | 8 | 100 | 9.4 (7.4) | 75 (59) |
| Stock diet ............. | 4 | 4 | 100 | 10.3 (8.0) | 41 (32) |

## II. Influence of fat nutrition on the second and subsequent generations

The growth of the 1st, 2nd, 3rd and 4th generations is recorded in Fig. 2, which gives the average curves for 2 males and 8 females, receiving in the one case butter, and in the other margarine or stock diet. These animals represent the parents of the following generation.

The curves of Fig. 2 cover a period of 3 months from the weaning period. After this time the animals were mated and were then no longer observed in this study.

*Table III.*

| Diet | No. of litters alive | No. of young alive | % of no. born (red.) | Average weight g | | Total weight of the offspring g |
|---|---|---|---|---|---|---|
| | | | | of the litters | per individual | |
| 3. generation at the age of 14 days. | | | | | | |
| Butter diet | 8 | 59 | 92.1 | 158.1 | 21.4 | 1265 |
| Marg. diet | 8 | 55 | 93.2 | 162.0 | 23.4 | 1296 |
| Stock diet | 4 | 32 | 100 | 161.3 | 20.2 | 645 |
| 3. generation at the age of 30 days. | | | | | | |
| Butter diet | 8 | 59 | 92.1 | 360.4 | 48.9 | 2883 |
| Marg. diet | 8 | 55 | 93.2 | 357.4 | 51.9 | 2859 |
| Stock diet | 4 | 32 | 100 | 316.8 | 39.6 | 1267 |

3. generation mated.

| Diet | Females bred | No. of litters born | per cent parturition | Average no. of young per litter | | No. of young of the 4. generation | |
|---|---|---|---|---|---|---|---|
| | | | | born | red. | born | red. |
| Butter diet | 8 | 8 | 100 | 10.4 | (8.0) | 83 | (64) |
| Marg. diet | 8 | 7 | 88 | 10.1 | (8.0) | 71 | (56) |
| Stock diet | 4 | 4 | 100 | 9.8 | (8.0) | 39 | (32) |

*Table IV.*

| Diet | No. of litters alive | No. of young alive | % of no. born (red.) | Average weight g | | Total weight of the offspring g |
|---|---|---|---|---|---|---|
| | | | | of the litters | per individual | |
| 4. generation at the age of 14 days. | | | | | | |
| Butter diet | 8 | 59 | 92.3 | 146.1 | 19.9 | 1169 |
| Marg. diet | 7 | 53 | 94.6 | 154.3 | 20.4 | 1080 |
| Stock diet | 4 | 28 | 87.5 | 143.0 | 20.4 | 572 |
| 4. generation at the age of 30 days. | | | | | | |
| Butter diet | 8 | 57 | 89.1 | 294.4 | 41.4 | 2355 |
| Marg. diet | 7 | 51 | 91.1 | 301.7 | 41.4 | 2112 |
| Stock diet | 4 | 28 | 87.5 | 271.3 | 38.7 | 1085 |

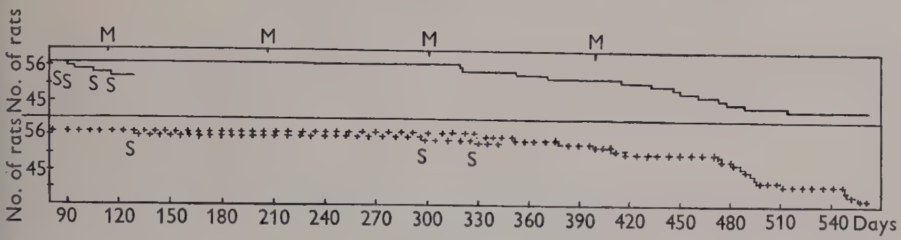

Fig. 3.

+ + + + Margarine diet — M = Mating

———— Butter diet — S = Substitution (comp. p. 39)

The tables I–IV give the detailed data concerning the fertility of the 1st, 2nd and 3rd generations respectively, namely

the number of females bred,
the number of litters born,
the percentage of parturition, calculated from the number of litters per females bred,
the average number of young per litter and
the number of young of the subsequent generation.

## III. Mortality of the first generation

In Fig. 3 we give the mortality of the female rats of the 1st generation, which received either butter or margarine diet.

*Group 1. Butter Diet*

| No. of rat | Sex | Age days | Symptoms |
|---|---|---|---|
| 49 129 | ♂ | 558 | steatosis organorum. |
| 49 158 | ♂ | 460 | bronchopneumonia. |
| 49 196 | ♂ | 471 | pneumonia abscedens pulm. sin. |
| 49 212 | ♂ | 443 | pneumonia abscedens pulm. dex. |
| 49 257 | ♂ | 463 | pneumonia abscedens bilateralis. |
| 49 268 | ♂ | 444 | bronchopneumonia bilateralis loborum inf. pulm. utriusque hepatisatio rubra pulm. dex. |
| 49 123 | ♀ | 475 | bronchopneumonia abscedens pulm. dex. et incipiens lobi sup. pulm. sin. |
| 49 197 | ♀ | 479 | abscessi multiplices pulm. dex.; diverticulum ilei sine stenosi. |
| 49 202 | ♀ | 430 | bronchopneumonia abscedens pulm. dex. |
| 49 230 | ♀ | 470 | bronchopneumonia; dilatatio cordis. |
| 49 223 | ♀ | 413 | abscessi multiplices pulm. dex. |
| 49 246 | ♀ | 566 | cysta dermoides cum pneumonia interstitialis; emphysema vicariens pulm. sin.; tumor lienis chronicus. |
| 49 267 | ♀ | 559 | bronchopneumonia incipiens pulm. sin.; steatosis organ. |
| 49 269 | ♀ | 443 | pneumonia abscedens toti pulm. dex. et lobi sup. pu. m. sin.; otitis media purulenta; steatosis hepatis; tumor lienis chronicus. |
| 49 200 | ♀ | 443 | anaemia; steatosis organ; abscessi pulm. sin. |
| 49 174 | ♀ | 448 | bronchopneumonia abscedens. |

## *Group II. MW (Margarine) Diet*

| No. of rat | Sex | Age days | Symptoms |
|---|---|---|---|
| 49 120 | ♂ | 485 | hypertrophia et dilatatio M. cordis; anaemia se. steatosis organ; rum tumor caudae necr. |
| 49 153 | ♂ | 558 | pneumonia abscedens chr. bilateralis cum exceptione lobi sup. pulm. sin.; tumor lienis induratus.<br>steatosis organorum. |
| 49 207 | ♂ | 500 | pneumonia abscedens bil. |
| 49 665 | ♂ | 448 | bronchopneumonia abscedens pulm. dex. |
| 49 098 | ♀ | 554 | hypertrophia et steatosis hepatis; necroses hepatis multipli. |
| 49 101 | ♀ | 574 | pneumonia abscedens bilateralis; steatosis organ. |
| 49 505 | ♀ | 480 | bronchitis et bronchopneumonia abscedens bilateralis. |
| 49 148 | ♀ | 471 | bronchopneumonia abscedens. |
| 49 163 | ♀ | 569 | pneumonia; steatosis organorum. |
| 49 168 | ♀ | 569 | abscessus hepatis cum peritonitide reactiva circumscripta; abscessus ovarii dex. |
| 491 80 | ♀ | 472 | pneumonia bilateralis; pleuritis seropurulenta; otitis media purulenta sin. |
| 49 190 | ♀ | 484 | pneumonia massiva pulm. dex. cum hepatisatione grisea partis inf. pulm. |
| 49 192 | ♀ | 493 | pneumonia abscedens dex. |
| 49 198 | ♀ | 571 | adhaesiones pleurales dex.; steatosis organ.; bronchopneumonia lobi sup. pulm. sin. |
| 49 201 | ♀ | 571 | neoplasma malign. pulm. dex. necrotisans et abscedens; pleuritis exudativa serosa bilateralis. |
| 49 225 | ♀ | 546 | bronchopneumonia diffusa bilateralis. |
| 49 227 | ♀ | 546 | bronchopneumonia diffusa bilateralis, partem abscedens et gangraenosa, cum pleuritise serofibrinosa. |
| 49 231 | ♀ | 455 | bronchitis fibrinosa; emphysema pulm. |
| 49 244 | ♀ | 490 | bronchopneumonia abscedens. |
| 49 247 | ♀ | 492 | bronchopneumonia abscedens; emphysema pulm. |

## *Group III. Stock Diet*

| No. of rat | Sex | Age days | Symptoms |
|---|---|---|---|
| 49 106 | ♂ | 460 | hepatisatio rubra pulm. sin. et pantis pulm. dex.; emphysema vic. cum oedema partis pulm. dex. |
| 49 134 | ♂ | 507 | pneumonia massiva dex.; emphysema pulm. sin. |
| 49 217 | ♂ | 466 | pneumonia bilateralis abscedens. |
| 49 911 | ♂ | 446 | bronchitis fibrinoso-purulenta cum emphysema pulm. sin.; pneumonia abscedens pulm. dex. |
| 49 142 | ♀ | 513 | bronchopneumonia et bronchitis diff. bilat. |
| 49 128 | ♀ | 562 | pneumonia pulm. dex.; emphysema pulm. sin. |
| 49 181 | ♀ | 559 | tumor parietis abdominis necroticus; bronchopneumonia incipiens bil. |
| 49 161 | ♀ | 572 | bronchopneumonia abscedens bil. |
| 49 208 | ♀ | 510 | bronchitis bilateralis abscedens; anaemia org. |
| 49 236 | ♀ | 522 | pneumonia abscedens pulm. dex. |
| 49 245 | ♀ | 490 | pneumonia caseoso-purulenta pulm. dex. cum adhaes. pleur.; hepatisatio rubra pulm. sin. |
| 49 248 | ♀ | 494 | bronchitis purul. et bronchopneumonia abscedens bilat. |
| 46 255 | ♀ | 550 | pneumonia abscedens max. gradu pulm. utriusque; emphysema vic. lobi inf. pulm. dex.; tumor lienis; steatosis organ. |
| 49 259 | ♀ | 510 | ileus chronicus cum adhaesione ilei et dilatatione intestini majoris gradu. |

Concerning the values for mortality we wish to mention, that the total number of rats in the series was maintained during 9 months in such a manner, that when a rat died it was substituted (S in the fig. 3) by another, treated in the same way as the dead one.

The autopsy was made and the observations given by Dr. med. LEO HELLER. We wish to express our sincere thanks to him for his kind and very valuable help.

## SUMMARY

From our previous investigation I–III we concluded that the nutritional and physiological value of the fat mixtures MW, which correspond to the margarine manufactured in Sweden during the period from October 1943 to November 1946 was in no way inferior to the value of butter.

Also for the experimental series here recorded a difference of significance between both sorts of fat in regard to the detailed data of fertility studied here has not been found.

A more complete discussion of these results will be given in connection with a review of the problem studied in our four investigations carried out on the influence of fat nutrition.

Stockholm, University, Vitamin Institute.

Tryckt den 4 januari 1951.

Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

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# The structure of some reaction products from acetylacetone and hydrogen sulphide

By ARNE BRÄNDSTRÖM

With 1 figure in the text

In a previous paper (1) the isolation of the compound $C_{10}H_{16}S_4$ m. p. 162.5–163° and an assumed compound $C_{10}H_{16}O_2S_2$ m. p. 138–139° from the reaction products of acetylacetone and hydrogen sulphide has been reported. The existence of the latter compound has now been established, and it is found to have the m. p. 138.2–138.4°, and another compound of the same formula $C_{10}H_{16}O_2S_2$ with m. p. 131.2–131.4° has been isolated.

The absorption spectra (Fig. 1) of these three compounds show very great similarities. From this it is evident that these compounds have similar structures. The absorption spectra of the three compounds from acetylacetone and that of tetraethenyl hexasulphide $C_8H_{12}S_6$ m. p. 224–225° are also very similar, which is in good agreement with the hypothesis (1) that the compounds $C_{10}H_{16}S_4$ and $C_8H_{12}S_6$ would have similar ring systems.

If the "adamantoid" (2) structure (I–IV) of these compounds, suggested by FREDGA (1, 2, 3), is adopted, there should be two possible compounds $C_{10}H_{16}O_2S_2$ (II, III), one of them containing two sulphur atoms in the same six-membered ring, the other, which must exist in two enantiomorphic forms, containing only one sulphur atom in each of the four six-membered rings.

According to the old structure set forth by FROMM and ZIERSCH (4), there should be three possible compounds $C_{10}H_{16}O_2S_2$ (II a, III a, and III b), one of them containing two sulphur atoms in the same four membered ring. The two others are cis-trans-isomers with one sulphur atom in each four-membered ring.

A way to decide which formula is the correct one for each compound offers the oxidation with potassium permanganate. FROMM and ZIERSCH (4) showed that the product $C_{10}H_{16}S_4$ (I or I a) was not oxidized to the corresponding sulphone, but cleft with the evolution of carbon dioxide. On the other hand, the compound $C_{14}H_{24}S_4$ (V or V a) from dimethylacetylacetone was easily oxidized to the compound $C_{14}H_{24}O_4S_4$. At last I have oxidized the compound $C_{12}H_{20}S_4$ (VI, VI a, VI b) from methylacetylacetone to the compound $C_{12}H_{20}O_6S_4$. For these differences FROMM and ZIERSCH's formulae cannot give any reasonable explanation. As there are very good reasons to believe that the angle between the two S-C-bonds in a sulphone is considerably smaller than in the corresponding thioether (5), the oxidation would decrease the strain and hence increase the stability of the compounds.

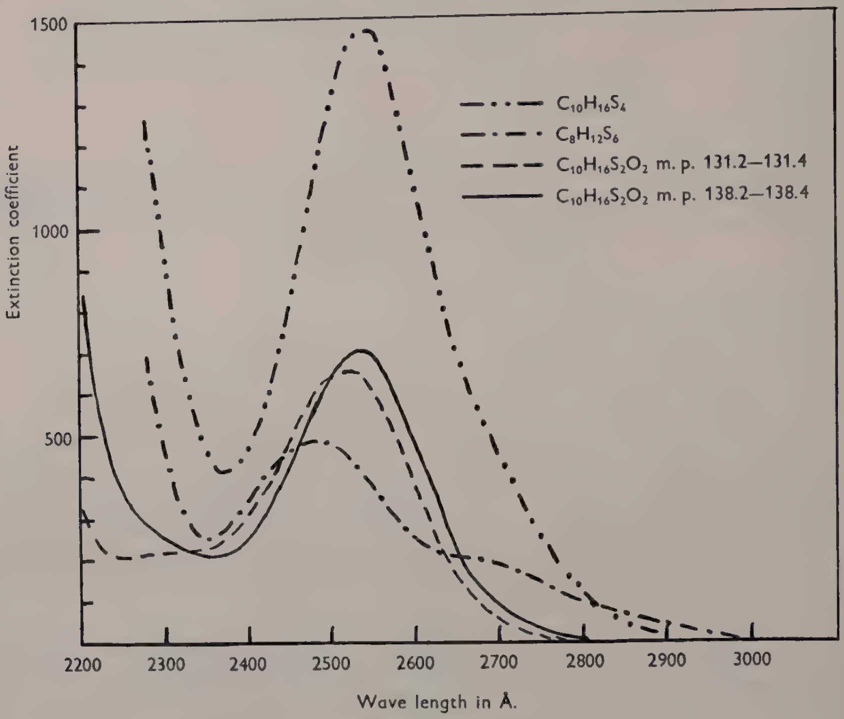
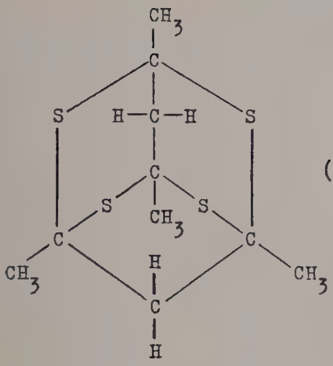


Fig. 1. Absorption spectra in hexane.

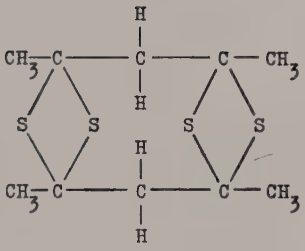
Quite different is the case if the "adamantoid" formulae are adopted. A compound of this structure is quite strainless but very rigid, and the valence angles of the sulphur cannot be appreciably smaller than the tetrahedral angle, without introducing a strain into the molecule. For this reason it seems to be very probable that only two sulphur atoms situated in different six-membered rings can be oxidized to sulphone groups without introducing a serious strain into the molecule. This is the case with the compound $C_{10}H_{16}O_2S_2$ m. p. 131.2–131.4°, which is oxidized to the disulphone $C_{10}H_{16}O_6S_2$, and for that reason must have formula III.

If for any reason two sulphur atoms belonging to the same six-membered ring are oxidized to sulphone groups, the molecule will be very strained, and the $CH_2$-groups in the β-positions to both sulphone groups would be much exposed to further oxidation, and the molecule will be cleaved if these groups are not protected. This is the case with the compounds $C_{10}H_{16}S_4$ and $C_{10}H_{16}O_2S_2$ m. p. 138.2–138.4°, which for that reason must have formulae I and II respectively.

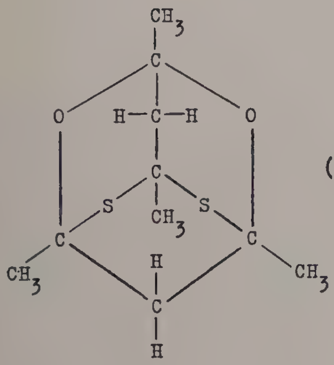
If the $CH_2$-groups are protected by substitution with alkyl groups as in the compound $C_{12}H_{20}S_4$ (VI) from methylacetylacetone and $C_{14}H_{24}S_4$ (V) from dimethylacetylacetone, this cleavage cannot be effected. In the case of the former compound, a trisulphone and in the latter a disulphone is obtained. That more sulphur atoms are not oxidized to sulphone groups may be due to sterical reasons.



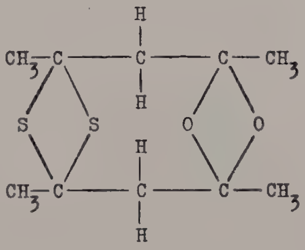
( I )



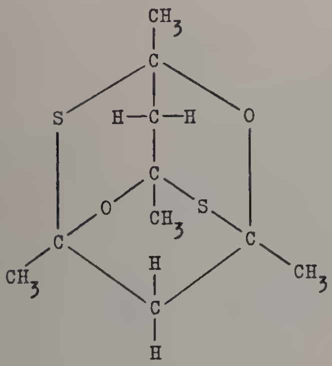
( Ia )



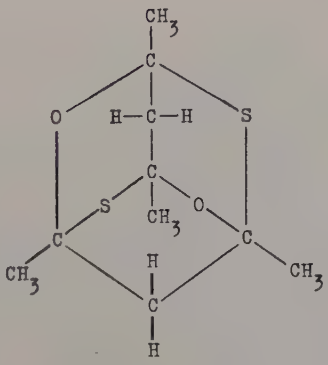
( II )



( IIa )



( III )



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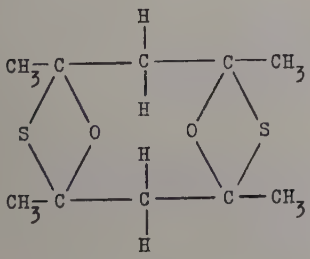

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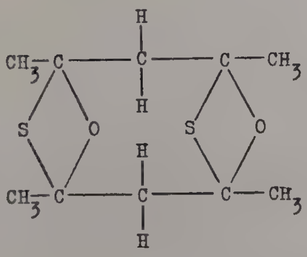
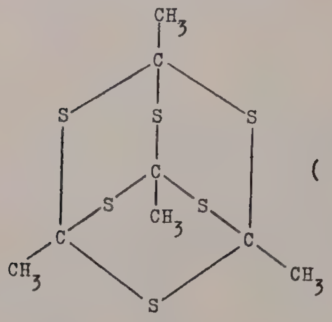
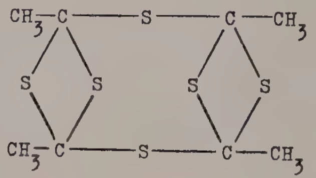


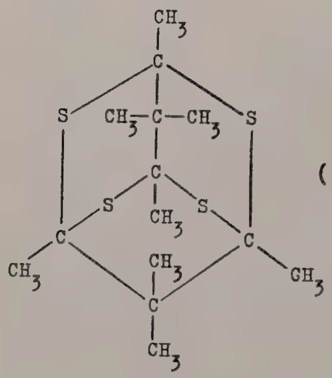
( IIIb )



( IV )



( IVa )



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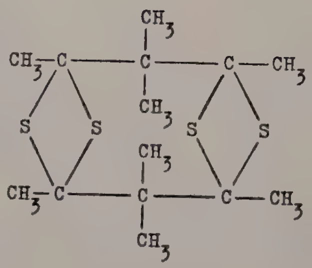

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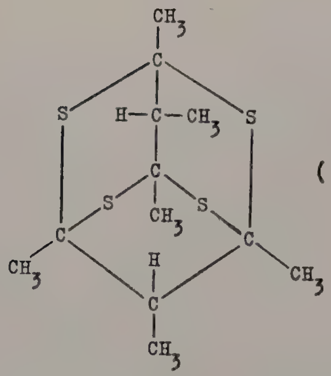

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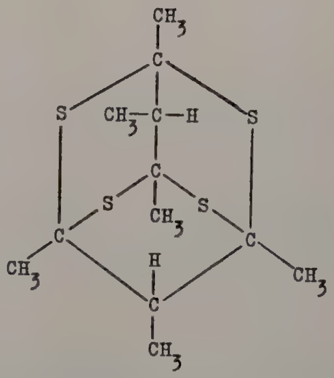
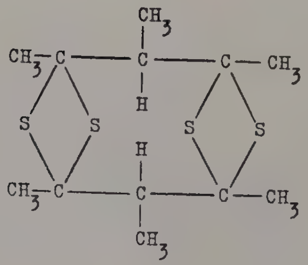
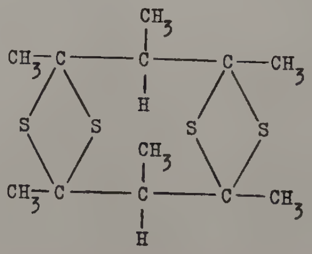


( VI )



( VIa )



( VIb )

The fact that a trisulphone is actually obtained from the compound $C_{12}H_{20}S_4$ (VI) is very interesting, and indicates that the cleavage of the compound $C_{10}H_{16}S_4$ (I) perhaps proceeds over the formation of a trisulphone. This indicates a certain difference between the dimeric dithioderivatives or β-diketones and the homologues of tetraethenyl hexasulphide (6).

Further evidence for the compound $C_{10}H_{16}O_2S_2$ m. p. 138.2–138.4° having formula II is that the absorption spectra of this compound reveals the best agreement with that of the compound $C_{10}H_{16}S_4$ (I), and that these two compounds yield a crystalline precipitate when treated with mercuric chlorid, but the compound $C_{10}H_{16}O_2S_2$ m. p. 131.2–131.4° does not yield any.

## Experimental

### The isolation of the compounds $C_{10}H_{16}O_2S_2$

A slow stream of hydrogen sulphide was passed into a 20 % solution of acetylacetone in concentrated hydrochloric acid at room temperature. At intervals, the gas stream was interrupted and the crystalline precipitate was filtered off. When no more crystals were formed, the product was washed with water and dried in the air.

25 g of the above product was treated with steam until the melted product resolidified, and only a minor quantity of solid substance passed over. The residue and the distilled product were filtered separately and dried in the air. The residue weighed 10.5 g and the distilled product 14.5 g.

**Compound $C_{10}H_{16}O_2S_2$ m. p. 138.2–138.4° (II):** The residue was recrystallized from ligroin (b. p. 60–70°). A first recrystallization yielded 5.5 g of a product melting at 139–142°, a second 4.3 g melting at 136–136.4°, and a third 3.7 g melting at 138.2–138.4° having a sulphur content of 27.67 %. The melting point and the sulphur content were not altered by further recrystallization, and this product represents the pure compound. From the mother liquors a further quantity of the compound can be obtained by recrystallization from ligroin.

The most characteristic property of this compound is, as mentioned before (1), the very sparing solubility in ligroin and benzene. On the other hand, it is a little more soluble in alcohol than the other compounds obtained from the reaction mixture.

If the ligroin is distilled off from the mother liquors, and the residue recrystallized from methanol five recrystallizations yield the pure compound $C_{10}H_{16}S_4$ m. p. 162.5–163° (I), which is the only product obtained at very low temperatures (1).

**Compound $C_{10}H_{16}O_2S_2$ m. p. 131.2–131.4° (III):** The steam distilled product was recrystallized from methanol, yielding the folloving fractions:

| Recrystallization | Sulphur content % | M. p. | Yield g |
|---|---|---|---|
| 1 | — | 100 —109° | 8.7 |
| 2 | 30.53 | 113 —121° | 6.5 |
| 3 | 29.11 | 116 —124° | 4.2 |
| 4 | 28.76 | 123 —127° | 3.3 |
| 5 | 28.34 | 126 —128.5° | 2.2 |
| 6 | 28.02 | 127.5—129.5° | 1.6 |
| 7 | 27.70 | 130.5—132° | 1.2 |
| 8 | 27.62 | 131.2—131.4° | 0.8 |

Further recrystallizations did not alter the melting point or the sulphur content. This product represents the pure compound $C_{10}H_{16}O_2S_2$ m. p. 131.2–131.4° (III). From the mother liquors a further quantity of the compound can be obtained by recrystallization from methanol.

This compound is confusingly similar to the compound $C_{10}H_{16}S_4$ (I) in appearance and solubility.

24.56 mg: 46.38 mg $CO_2$, 15.20 mg $H_2O$. — 28.57 mg: 57.53 mg $BaSO_4$. — 27.45 mg: 6.396 ml 0.07396-*N* NaOH.

$C_{10}H_{16}O_2S_2$ (232.4) calc. C 51.69 H 6.94 S 27.60
found » 51.53 » 6.93 » 27.66, 27.62

0.1494, 0.3020, 0.4422 g in 15.00 g benzene: E = 0.117°, 0.232°, 0.347°

M. W. calc. 232, found 219, 224, 218.

If the methanol is distilled off from the mother liquors, and the residue recrystallized from ligroin, a quantity of the compound $C_{10}H_{16}O_2S_2$ m. p. 138.2–138.4° (II) can be obtained.

### The oxidation products

**Disulphone $C_{10}H_{16}O_6S_2$:** 0.2 g of the compound $C_{10}H_{16}O_2S_2$ m. p. 131.2–131.4° (III) was dissolved in benzene and shaken for 1 hour with an excess of potassium permanganate dissolved in 10 % sulphuric acid. Sodium bisulphite was added until the mixture was discoloured. The benzene solution was separated and filtered in order to remove a minor quantity of a slightly coloured precipitate. The benzene was then driven off, and the residue recrystallized from alcohol, yielding the colourless disulphone with dec. p. about 250°. This compound is very sparingly soluble in alcohol, but readily soluble in benzene.

36.51 mg: 54.18 mg $CO_2$, 16.84 mg $H_2O$. — 35.24 mg: 7.070 ml 0.06725-*N* NaOH.

$C_{10}H_{16}O_6S_2$ (296.4) calc. C 40.53 H 5.44 S 21.64
found » 40.50 » 5.16 » 21.63.

When a small quantity of the compound $C_{10}H_{16}O_2S_2$ m. p. 138.2–138.4° (II) was treated in the same way a vigorous reaction set in with the evolution of carbon dioxide.

**Trisulphone $C_{12}H_{20}O_6S_4$:** A small quantity of the compound $C_{12}H_{20}S_4$ (V) from methylacetylacetone (1) was treated in the way mentioned above, giving a very good yield of the colourless trisulphone with dec. p. about 260°. The solubility of this compound is nearly the same as that of the disulphone mentioned above.

22.17 mg: 30.03 mg $CO_2$, 9.76 mg $H_2O$. — 18.89 mg: 5.770 ml 0.06725-*N* NaOH.

$C_{12}H_{20}O_6S_4$ (388.5) calc. C 37.09 H 5.19 S 33.01
found » 36.96 » 4.94 » 32.93.

## SUMMARY

Two compounds of the formula $C_{10}H_{16}O_2S_2$ have been isolated from the reaction products of acetylacetone and hydrogen sulphide at room temperature.

A very strong argument for the "adamantoid" structure of the dimeric dithioderivatives of β-diketones has been obtained.

The formulae of each compound $C_{10}H_{16}O_2S_2$ have been advanced.

Sulphones of the compound $C_{10}H_{16}O_2S_2$ m. p. 131.2–131.4° and the dimeric dithio-3-methyl-acetylacetone have been prepared.

The investigation is continued along different lines.

This investigation has been supported by a grant from Statens Naturvetenskapliga Forskningsråd which is gratefully acknowledged.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, October 1950.

REFERENCES: 1. **A. Fredga** and **A. Brändström,** Arkiv f. Kemi, Mineral. Geol. *26 B* N:o 4 (1948). — 2. **A. Fredga,** Arkiv f. Kemi, Mineral. Geol. *25 B* N:o 8 (1947). — 3. **A. Fredga** and **A. Brändström,** Arkiv f. Kemi *1* N:o 22 (1949). — 4. **E. Fromm** and **P. Ziersch,** Ber. *39,* 3599 (1906). — 5. **A. Lüttringhaus** and **K. Buchholz,** Ber. *72,* 2057 (1939). — 6. **A. Fredga** and **H. Bauer,** Arkiv f. Kemi *2* N:o 3 (1950).

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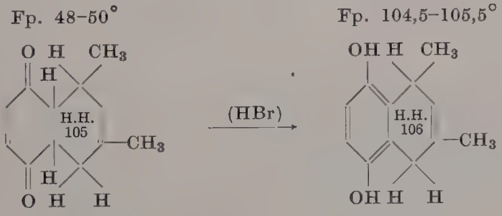
# Dien-Reaktionen. II

Von Hans v. Euler, Hans Hasselquist und Armin Glaser

In einer vorhergehenden Mitteilung[1] wurden Derivate des aus 1,3-Dimethylbutadien und p-Benzochinon entstehenden Ausgangsproduktes HH 105 beschrieben. Die bei der Umsetzung HH 105–HH 106 eintretende starke Wärme-Entwicklung wurde wegen ihres theoretischen Interesses durch orientierende Messungen bestimmt.



Bei der Konzentration von 600 mg der Sbst. HH 105 in 2 ccm Eisessiglösung (enthaltend 130 γ HBr) und bei einer Anfangstemperatur von 22,6° und einer Abstrahlung entsprechend einem Temperaturfall von 2,9° per Minute berechnete sich die erreichte (korrigierte) Temperatur zu 53,5°; die Temperaturerhöhung des Systems betrug $53{,}5 - 22{,}6 = 30{,}9°$. Pro Mol berechnet sich für die eingehaltenen Bedingungen die Umwandlungswärme $105 \rightarrow 106$ zu rund 21 K cal.

Um die Wärmeabstrahlung zu kompensieren wurde in einem anderen Versuch die Lösung von 600 mg Sbst. HH 105 in 2 ccm Eisessig auf 9,0° abgekühlt (eine weitere Abkühlung liess sich nicht durchführen ohne dass die Sbst. 105 ausfiel) und mit einem Tropfen HBr-Eisessig versetzt. Die Temperatur stieg anfangs rel. langsam, bei 41,3 war das Maximum erreicht. Die Unterkühlung betrug 14,2°.

| Aussentemp. | Anfangstemp. | Höchste Temp. | Temp.-Zunahme | Erhöhung über Zimmertemp. |
|---|---|---|---|---|
| 23,2° | 9,0° | 41,3° | 32,3° | 18,1° |

Bei einem Wärmewert der Apparatur von 2,1 cal pro Grad berechnet sich hieraus eine Wärmemenge von 67,8 cal für 600 mg HH 105. Für ein Mol berechnet sich die entwickelte Wärmemenge zu 21,0 K cal, in vollständiger Übereinstimmung mit dem in obigem Versuch erhaltenen Wert.

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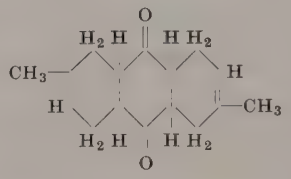
[1] Euler u. H. Hasselquist, Dieses Arkiv 2, Nr. 23 (1950). — Nr. I dieser Serie.

Analoge Messungen wurden an weiteren, aus verschiedenen Butadienen[1] und Benzochinon dargestellten, partiell hydrierten Naphtochinonen angestellt. Ihre Umwandlung in die entspr. Naphtochinone gelingt (mit HBr) auch ohne Eisessig. Man beobachtet dann beim Erhitzen über den Schmpt. eine lebhafte Reaktion, nach deren Verlauf die Masse erstarrt.

## I. Isopren und p-Benzochinon

Euler und Josephson hatten die erste Diensynthese[2] am System Isopren und p-Benzochinon durchgeführt. Bei dem von ihnen angewandten Überschuss von Isopren wurde damals nicht Mono-isopren-chinon sondern Bis-isopren-chinon (I) erhalten,



(I)

und zwar, wie später Diels und Alder[3] zeigten, ein Gemisch (A + B) der bereits umgelagerten Strukturisomeren.

Zur Darstellung des Mono-isopren-chinons[4] haben wir 1 Tl. Benzochinon mit 1,5 Tln. Isopren 1 Stunde zum Sieden erhitzt und über Nacht bei Zimmertemp. stehen lassen. Die ölige Masse erstarrte beim Anreiben zu schwach gelblichen Nadeln, die, umkrystallisiert, bei 85°–86° konstant schmolzen. (Sbst. 103 b.)

### Umlagerung des Mono-isopren-chinons durch HBr

Die Umlagerung wurde in der gleichen Weise vorgenommen, die früher[5] für die Sbst. 105 beschrieben wurde. Das unter starker Wärme-Entwicklung entstehende Produkt zeigt nach Umkrystallisieren den Schmpt. der Sbst. 104 b.

### Wärmetönung der Umlagerung 103 b–104 b

Die früher kurz beschriebene, primitive Apparatur wurde durch Bestimmung der Temperaturerhöhung bei Neutralisation von Eisessig mit 5 norm. wässriger Natronlauge geeicht.

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[1] Sämtliche hier verwendete Butadiene sind uns von Herrn Dr. Walter Reppe, Leiter d. Forschung d. Bad. Anilin- und Soda-Fabrik, Ludwigshafen/Rhein, freundlichst überlassen worden. Wir sprechen Herrn Direktor Reppe auch hier unseren verbindlichsten Dank aus.

[2] Euler u. K. Josephson, Chem. Ber. 53, 822 (1920).

[3] O. Diels u. K. Alder, Chem. Ber. 62, 2337 (1929).

[4] Ein Mono-isopren-chinon ist in den Patentschriften der I. G. Farbenindustrie A. G. (F. P. 677 296 und 677 781: 1929) (Darstellung bei 50° unter Druck) kurz erwähnt. Siehe auch Chem. Zbt. 1930, II 809.

[5] Euler u. H. Hasselquist, Dieses Arkiv 2, Nr. 23 (1950).

Bei der Umlagerung von 555,2 mg Mono-isopren-chinon (unter Zusatz einer kleinen Menge HBr) stieg die Temperatur um 21,9°. Die Temperaturänderung von 1° entspricht in unserem Apparat einer entwickelten Wärmemenge von 2,7 cal. Der aus der Abstrahlungskurve entnommene mittlere Strahlungsverlust entspricht einem Temperaturfall von 1,8° per Minute. Ab etwa 39,8° stimmt die Abstrahlungskurve mit der Umlagerungskurve überein. Daraus ergibt sich die Endtemperatur, die ohne Abstrahlung erreicht würde, zu $39{,}8 + 9 \cdot 1{,}8 = 56°$ (9 Min. ist die bis zum Erreichen der Temp. 39,8 verstrichene Zeit). Da der Ausgangspunkt bei 24,1° lag betrug die Temperaturänderung $56 - 24{,}1 = 31{,}9°$.

Der Wasserwert des Systems entsprach 2,1 cal pro Grad. Somit berechnet sich die Wärmeentwicklung für 555,2 mg Sbst. 67,3 cal oder per Mol 21.0 K cal.

## II. 1,4-Dimethyl-butadien und p-Chinon

2 g Chinon wurden mit 4 g 1,4-Dimethyl-butadien[1] auf dem Wasserbad erwärmt bis alles Chinon gelöst war. Durch die Reaktionswärme hielt sich die Lösung noch eine Zeitlang heiss. Nach ca. 2-stündigem Stehen wurde das überschüssige Butadien abgedampft; der gekühlte, ölige Rückstand erstarrte beim Anreiben zu einer breiigen Masse; Ausbeute 2,5 g, Schmpt. des abgepressten Rohproduktes 53°–56°. Die durch Umkrystallisieren aus Petroläther erhaltenen grünlichen prismatischen Nadeln schmolzen bei 58°–59,5° (Sbst. 114). Löslich in allen gewöhnlichen organischen Lösungsmitteln, unlöslich in Wasser.

Mol. Gew.: 67,5 mg Sbst. 114 ergaben in 2,108 g Eisessig eine Gefrierpunkts-Erniedrigung von 0,660°. Hieraus

| | |
|---|---|
| Mol. Gew. gef. | 189,2 |
| ber. für $C_{12}H_{14}O_2$ | 190,2 |

### Umlagerung der Sbst. HH 114 durch HBr in Eisessig

600 mg der Sbst. HH 114 in 2 ml Eisessig gelöst und mit ganz wenig HBr versetzt.[2] Starke Wärme-Entwicklung (siehe unten). Das bei der Abkühlung ausfallende Produkt (insgesamt 570 mg weisse Nadeln vom Schmpt. 166°–167°) besass, aus Benzol umkrystallisiert, den Schmpt. 167°–168°. Sbst. HH 115.

Mol. Gew. durch Gefrierpunkts-Erniedrigung in Eisessig

| | |
|---|---|
| Mol. Gew. gef. | 200,1 |
| ber. für $C_{12}H_{14}O_2$ | 190,2 |

| Analysen | 114 | | 115 | |
|---|---|---|---|---|
| Berechnet für $C_{12}H_{14}O_2$ | C = 75,76 | H = 7,42 | C = 75,76 | H = 7,42 |
| Gefunden | 75,13 | 7,38 | 75,61 | 7,80 |

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[1] Siehe hierzu auch B. ARBUSOW u. S. M. SPEKTERMANN, Trns. Kirovs Inst. chem. Techn. Kazan. 8, 21; 1940. — Chem.Zbt. 1942, I, 481/82. 1942. Soweit aus dem Referat ersichtlich stimmen die Angaben der russischen Forscher mit unseren Ergebnissen nicht überein.

[2] Die Mitwirkung des Katalysators HBr (auch HCl und vermutlich auch andere starke Säuren sowie Basen sind verwendbar) ist für das Zustandekommen der Umwandlung erforderlich. Von der Konzentration des Katalysators ist aber die Wärmetönung nur wenig abhängig.

### Wärmetönung der Umlagerung HH 114–115

Innerhalb 45 Sekunden war die höchste Temperatur, 52°, erreicht.

Der Abstrahlungsfaktor konnte zu 2,5° per Minute bestimmt werden. Als Wasserwert wurde 2,11 cal pro Grad in Rechnung gesetzt.

Bei 51° stimmt die Wärmeentwicklungs-Kurve mit der Abstrahlungskurve überein und zwar betrug die Zeit 2 Minuten. Ohne Abstrahlung wäre eine Temperatur von $51 + 2 \cdot 2{,}5 = 56°$ erreicht worden. Der Anfangspunkt lag bei 21,8°, also betrug die Temperatursteigerung 34,2°. Die entwickelte Wärmemenge berechnet sich zu

$$34{,}2 \cdot 2{,}11 = 72{,}1 \text{ cal.}$$

Für 1 Mol berechnet sich die entwickelte Wärmemenge zu 22,8 K cal.

## III. 1-Äthyl-butadien und p-Chinon

2 g Chinon mit 2,5 g 1-Äthyl-butadien auf dem Wasserbad erwärmt und die Mischung 1 Stunde bei schwachem Sieden gehalten, wobei ein Teil des Butadien-Überschusses verdampfte. Der so erhaltene ölige Rückstand mit Petroläther gerieben. Die ausfallenden Krystalle abgesaugt (Ausbeute 2 g). Nach 4-maligem Umkrystallisieren aus Petroläther war der Schmpt. konstant bei 54°–56° Derbe, schwach gelblich-grünliche Krystalle. HH 116.

### Umlagerung der Sbst. HH 116

600 mg der Sbst. 116 in 2 ml Eisessig gelöst und mit ganz wenig HBr versetzt. Reaktion unter starker Wärme-Entwicklung (siehe unten). Das Produkt liess sich mit der 4-fachen Menge Wasser ausfällen. Ausbeute 500 mg weisse Nadeln, welche, aus Benzol umkrystallisiert, bei 122°–124° schmolzen. HH 117.

| Analysen | 116 | | 117 | |
|---|---|---|---|---|
| Berechnet für $C_{12}H_{14}O_2$ | C = 75,76 | H = 7,42 | C = 75,76 | H = 7,42 |
| Gefunden | 75,60 | 8,61 | 75,46 | 9,91 |

### Wärmetönung der Umlagerung HH 116–117

Versuchsanordnung wie bei der Bestimmung der Wärmetönung 114–115. Der Temperaturanstieg erreichte innerhalb 30 Sekunden sein Maximum. Die Ausgangstemperatur war 20,7°, das Maximum lag bei 53,6°. Unkorrigiert beträgt die Temperaturerhöhung 32,9°, korrigiert 33,6, Differenz somit 0,7°. Bei Annahme des Wasserwertes des Systems zu 2,1 cal pro Grad berechnet sich die entwickelte Wärmemenge unter Einführung der Korrektion zu (70,89) rund 70,9 cal pro 600 mg oder per Mol zu rund 22 K cal.

Zum Vergleich mit den Wärmetönungen der hier studierten Umwandlungen wurde mit der gleichen Apparatur und Versuchsanordnung die durch Neutralisation bestimmter Säuren- und Basen-Mengen hervorgerufene Temperatursteigerung gemessen.

| | | Anfangstemp. | Höchste Temp. | Temp.-Zunahme |
|---|---|---|---|---|
| Vers. I. 5,0 n. NaOH + 5,0 n. HCl | | | | |
| | Vers. I a | 24,7 | 54,6 | 29,9 |
| | b | 24,5 | 54,3 | 29,8 |
| Vers. II. 5,9 n. NaOH + 5,9 n. HCl | | | | |
| | Vers. II a | 25,5 | 61,4 | 35,9 |
| | b | 23,9 | 60,1 | 36,2 |

Die Temperatur-Zunahme von 29,85° in unserer Anordnung entspricht der Neutralisation von 0,005 Mol NaOH durch 0,005 Mol HCl. Bei den Umlagerungsversuchen kamen 600 mg der partiell hydrierten Naphtochinone (Mol. Gew. für $C_{12}H_{14}O_2 = 190$) = 0,0035 Mol zur Anwendung.

## IV. 1-Methoxy-butadien und p-Chinon

1 g Chinon wurde mit 1,8 ml 1-Methoxy-butadien[1] vorsichtig auf dem Wasserbad erwärmt. Beinahe unmittelbar setzte die Reaktion ein, die ohne äussere Wärmezufuhr weiter verlief, wobei sich die Lösung schwach gelb färbte. Nach dem Erkalten fiel das Reaktionsprodukt in Form gelbgrünlicher Prismen aus, welche mit wenig Petroleumäther gewaschen wurden. Ausbeute 1,5 g; Schmpt. 76°–79°. Durch Umkrystallisieren aus Benzol-Petroleumäther wurde die Sbst. HH 120 mit konstantem Schmpt. 79°–79,5° erhalten. Sie ist löslich in warmem Petroleumäther, im übrigen in allen gewöhnlichen organischen Lösungsmitteln.

### Umlagerung HH 120 → HH 121 durch HBr in Eisessig

750 mg Sbst. HH 120 in 0,5 ml Eisessig wurden mit einem mit HBr-gesättigtem Eisessig benetzten Glasstab in Berührung gebracht. Durch die sofort eintretende heftige Reaktion geriet der Eisessig ins Sieden, wobei das Umlagerungsprodukt in Form gelblichgrüner Stäbchen auskrystallisierte. Das abfiltrierte, mit Eisessig ausgewaschene Rohprodukt, 400 mg, schmilzt nach Umkrystallisieren aus Eisessig konstant bei 204,5°–205° (Rotbraunfärbung). Die reine Sbst. HH 121 ist leichtlöslich in Aceton, Äther, Alkohol und in warmem Eisessig, wenig löslich in Benzol.

| Analysen | 120 | | 121 | |
|---|---|---|---|---|
| Berechnet für $C_{11}H_{12}O_3$ | C = 68,73 | H = 6,29 | C = 68,73 | H = 6,29 |
| Gefunden | 67,24 | 6,32 | 74,63 | 5,03 |
| Berechnet für $C_{10}H_8O_2$ (Siehe Seite 7) | | | 74,99 | 5,04 |

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[1] Siehe hierzu über die Konstitution der Dienaddukte an 2-Methoxy- und 1-Acetoxy-butadien: H. FIESSELMANN, Angew. Chemie, 62, 344 (1950).

### Wärmetönung der Umlagerung HH 120–HH 121

Die Anordnung war dieselbe wie in den vorigen Versuchen. 606 mg HH 120 (entspr. 600 mg HH 105) wurden in 2 ccm Eisessig gelöst und mit einem Tropfen HBr in Eisessig versetzt.

| | Anfangstemp. | Höchste Temp. | Temp.-Zunahme |
|---|---|---|---|
| Versuch a | 22,1 | 81,0 | 58,9 |
| » b | 30,3 | 79,5 | 59,2 |

Wärme-Entwicklung pro Mol für 606 mg: beob. 123,7 u. 124,3 cal.
ber. 39,2 u. 39,4 K cal

## V. 1-Äthoxy-butadien und p-Chinon

1 g p-Chinon wurden mit 1,5 ml 1-Äthoxy-butadien versetzt. Nach weniger als 1 Minute geriet die bei 20° stehende Mischung durch lebhafte Reaktion ins Sieden. Nach Abkühlung im Kälteschrank rief Reiben mit einem Glasstab das Auskrystallisieren gelblichgrüner Blättchen hervor (Ausbeute: 1,15 g von Schmpt. 46°–47°). Aus Petroläther umkrystallisiert schmelzen die Tafeln konstant bei 53°–54°. Die Sbst. 122 ist löslich in warmem Petroleumäther und ziemlich leicht löslich in allen gewöhnlichen organischen Lösungsmitteln.

### Umlagerung der HH 122–HH 123

500 g Sbst. 122 in 0,5 ml Eisessig gelöst und mit einer Spur HBr versetzt gerät in stürmische Reaktion unter Kochen der Lösung. Nach Abkühlen der Mischung krystallisiert das Umlagerungsprodukt in Form gelbgrüner, spitziger Prismen aus. Nach Waschen mit etwas Eisessig schmilz das Rohprodukt (400 mg) bei 203°–207° unter Braunfärbung. Durch Umkrystallisierung aus Eisessig wird das reine Produkt vom Schmpt. 204,5°–205° erhalten. Diese Sbst. 123 ist leichtlöslich in Aceton und in Alkohol, löslich in warmem Eisessig, wenig löslich in Benzol. An der Luft oxydiert sich die Sbst. 123 zu einer grünvioletten Chinhydron-artigen Verbindung.

| Analysen | 122 | | 123 | |
|---|---|---|---|---|
| Berechnet für $C_{12}H_{14}O_3$ | C = 69,88 | H = 6,84 | C = 69,88 | H = 6,84 |
| Gefunden | 69,95 | 6,96 | 74,29 | 5,12 |
| Berechnet für $C_{10}H_8O_2$ | | | 74,99 | 5,04 |

### Wärmetönung der Umlagerung HH 122–HH 123

648 mg H 122 (entspr. 600 mg HH 105) wurden im 2 ccm Eisessig gelöst und mit einem Tropfen HBr in Eisessig versetzt.

| | Anfangstemp. | Höchste Temp. | Temp.-Zunahme |
|---|---|---|---|
| Versuch a | 21,0 | 77,7 | 56,7 |
| » b | 20,6 | 79,1 | 58,5 |

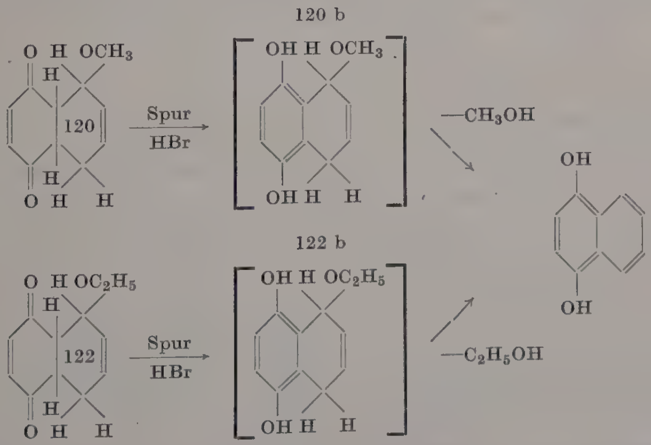
Wärme-Entwicklung pro Mol für 684 mg: beob. 119,1 u. 122,8 cal
ber. 37,9 u. 39,0 k cal

Wie aus den Angaben S. 53 u. 54 ersichtlich, sind die Substanzen HH 121 und HH 123 nicht die erwarteten Umlagerungsprodukte, vielmehr erwiesen sich die beiden Produkte durch ihren Schmpt. 204°–205° und ihre übrigen Eigenschaften als identisch, und eine Bestimmung des Mischschmelzpunktes zeigte keine Depression.

Um die Struktur der beiden Stoffe sicherzustellen wurden noch folgende Versuche ausgeführt:

I. Die Substanzen HH 121 sowie HH 123 wurden mit konz. $H_2SO_4$ und Essigsäureanhydrid 5 Minuten erhitzt. Nach Abkühlung und Verdünnung mit Wasser fiel ein Öl aus, das beim Reiben krystallisierte. Abfiltriert und mit Wasser gewaschen zeigte das Rohprodukt den Schmpt. 124°–126°. Durch Umkrystallisieren aus Essigsäure stieg der Schmpt. und wurde konstant bei 127°–128°. Er stimmt überein mit dem in der Literatur für 1,4 Diacet-oxy-naphtalin angegebenen.

II. Reines α-Naphtochinon (Schmpt. 125°) wurde in 4-n HCl aufgeschlemmt und mit Zinkpulver auf dem Wasserbad erwärmt. Nach Schluss der Wasserstoffentwicklung wurde die warme Lösung filtriert. Aus dem Filtrat schied sich beim Abkühlen 1,4-Dioxy-naphtalin in Form langer Nadeln ab, Schmpt. 203°–204°. Ein Mischschmelzpunkt mit den Substanzen HH 121 und HH 123 gab keine Depression.
Nach allen hier angegebenen Versuchen und Analysen-Resultaten sind somit beide Substanzen, 121 und 123 identisch mit *1,4 Dioxy-naphtalin.*



Bei der Umlagerung der beiden aus den Oxyalkyl-butadienen entstandenen hydrierten Naphtochinonen findet also unter Abspaltung von Alkohol eine Aromatisierung statt.

Während die Zusammensetzung der Substanzen 121 und 123 mit der für Dioxy-naphtalin berechneten gut übereinstimmt, weicht ihr Schmpt. stark von den in der Litteratur für Dioxy-naphtalin angegebenen ab, welche auffallenderweise auch unter sich wenig übereinstimmen:

| | |
|---|---|
| GROVES, Annalen, 167, 359 (1872) | 176° |
| PLIMTON, Soc. 37, 635 | 173° |
| RUSSIG, J. prakt. Ch. (2) 62, 32 (1900) | 176° |
| A. WOLFF, Annalen, 299, 278 (1913) | 192° |
| ELADIO OLAY CABAL[1], Rev. Acad. Ciencias 32, 384 (1935) | 172°—173° |

Wir haben deswegen unsere Substanzen 121 und 123 noch in folgender Weise durch Oxydation zu Naphtochinon identifiziert: Dieselben wurden in Äther gelöst und mit einer wässrigen Lösung von $CrO_3$ geschüttelt. Die Ätherschicht, welche sich dabei gelb färbte, wurde mit Natriumsulfat entwässert und zur Trockne verdampft. Die erhaltene Krystallmasse sublimierte bei etwa 110°. Das gelbe krystallisierte Produkt schmolz bei 125°. Die Mischung mit $\alpha$-Naphtochinon (Schmpt. 125°) ergab keine Depression.

Um das bei der Umlagerung von HH 122 vermutlich auftretende Zwischenprodukt 122 b zu isolieren wurden 100 mg Sbst. 122 in 10 ml Benzol gelöst und mit einer Spur HBr versetzt. Die ausfallenden filzigen Nadeln erwiesen sich als 1,4-Dioxy-naphtalin. Das Filtrat derselben wurde mit wenig Wasser ausgeschüttelt; durch Zusatz von Jodjodkalium und Alkali trat Jodoform-Geruch auf, der das bei der Umlagerung entstandene Äthanol anzeigte. Eindunsten der Benzollösung ergab nur das Ausgangsmaterial, kein Zwischenprodukt.

Auch bei Wiederholung des Versuchs bei 0° sowie bei —20° in Eisessig bezw. in Benzol konnte kein Zwischenprodukt erhalten werden.

Die Umwandlung, d. h. die unter Elektronenverschiebung stattfindene überführung des angulär gestellten H-Atoms auf das Carbonyl-Sauerstoff-Atom des Chinon-Kernes, die von der erheblichen Entwicklung einer Resonanz-Energie begleitet ist, führt zur Stabilisierung des Moleküls und verläuft — so weit unsere Erfahrung reicht — vollständig und ist irreversibel.

Die Messungen der Wärmetönungen der studierten Umlagerung sind in folgender Tabelle zusammengestellt:

| Butadien | Bezeichn. d. Umlager. im Text | Mol. Gew. | Schmpt. — Diff. | k cal[2] pro Mol |
|---|---|---|---|---|
| 2-Methyl | 103 b—104 b | 176 | 166 —86= 80 | 21,0 |
| 1,3-Dimethyl | 105 —106 | 190 | 105 —49= 56 | 21,0 |
| 1.4- » | 114 —115 | 190 | 167 —59=108 | 22,8 |
| 1-Äthyl | 116 —117 | 190 | 123 —55= 68 | 22,0 |
| 1-Methoxyl | 120 —121 | 192 | 204,5—79=125,5 | 39,3 |
| 1-Äthoxyl | 122 —123 | 206 | 204,5—54=150,5 | 38,5 |

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[1] In der Arbeit von ELADIO OLAY CABAL (Rev. Acad. de Ciencias, Madrid, 32, 384; 1935) wird auch die Bildung einer alelotropen Transformation des 1,4-Dioxy-naphtalins mitgeteilt.

[2] Es ist in Aussicht genommen, die Wärmetönungen durch Anwendung verfeinerter Apparatur genauer zu ermitteln. Weiterhin werden die Umwandlungswärmen mit den Diffe-

Bemerkenswert is hier, dass die Derivate von 1-Methoxy- und 1-Äthoxybutadien bedeutend höhere, fast doppelt so grosse Umwandlungswärmen ergeben als die Derivate der übrigen Butadiene und also HH 120 u. 122 einen noch viel höheren Energieinhalt besitzen als 105. Wie aus den Angaben S. 55 hervorgeht, handelt es sich bei den Oxyalkyl-dienen nicht um reine Umwandlungen, sondern auch um die damit verbundene Abspaltung der Oxymethyl- bezw. Oxäthyl-Gruppen.

Auch die *Geschwindigkeit* der Übergänge 120 → 121 und 122 → 123 ist besonders gross.

Auf die bei diesen Reaktionen möglicherweise auftretenden isomeren oder mesomeren Formen von partiell hydrierten Oxy-naphtochinonen kommen wir an anderer Stelle zurück.[1]

Stockholm, Universität, Institut f. organ.-chem. Forschung.

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renzen der Verbrennungswärmen der partiell hydrierten Naphtochinone und ihre Umwandlungsprodukte zu vergleichen sein. Auch die Bildungsreaktion der partiell hydrierten Naphtochinone verläuft unter starker Wärmeentwicklung. Es gelingt aber nicht, die Synthese direkt zu den Hydrochinonen zu leiten.

[1] Siehe hierzu auch E. C. Cabal, l.c. — Kurt H. Meyer, Ann. 379, 37; 1910. — Thiele, Ann. 306, 129; 1903.

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# New mononitriles of some dicarboxylic acids

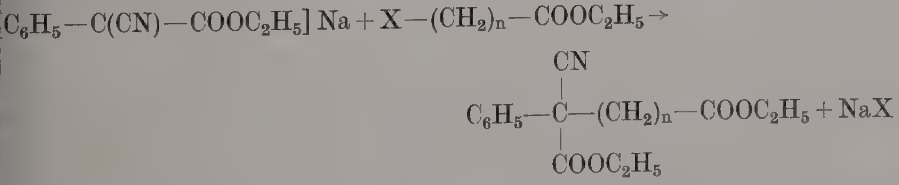
By SIGVARD WIDEQVIST

The cyano acids described in the present publication, except phenylcyano-cetic acid, have not been prepared before.

Phenylcyanoacetic acid was first prepared by HESSLER (1) by saponification f its ethyl ester by alkali. The crude product melted at about 62—65°. When he acid was recrystallized from ether-ligroin, the melting point was raised bout 10°. After two recrystallizations from hot benzene and drying in vacuo ver sulfuric acid the substance melted sharply at 92° C. The equivalent weight f the low-melting crude product, determined by means of its silver salt, was, owever, that of phenylcyanoacetic acid, $C_9H_7O_2N$.

The present author found, that the acid, after having been recrystallized wice from benzene and once from chloroform, melted sharply at 74—75°. etermination of the equivalent weight by titration with sodium hydroxide in-icated that the formula of the acid was $C_9H_7O_2N + H_2O$. Determination of arbon and hydrogen gave the same result. After drying *in vacuo* over phos-horous pentoxide the melting point was raised to 91—92°. Analysis of the ried product showed that its composition was $C_9H_7O_2N$. The acid $C_9H_7O_2N$, $H_2O$ is a real hydrate and not identical with the isomeric phenylmalonamic cid. This was proved by synthesis of the latter substance. The amic acid elts at 123—124° and is decarboxylized to phenylacetamide. Phenylcyano-cetic acid hydrate, on heating, gives benzyl cyanide.

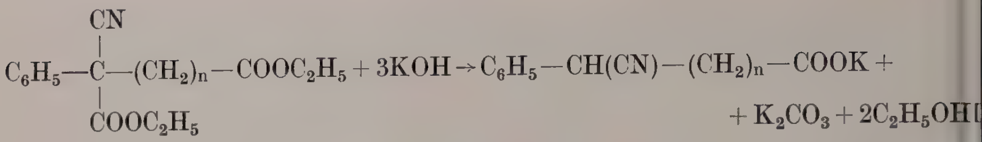
The homologues of phenylcyanoacetic acid are easily prepared according to a ethod previously described by the author (2), (3). The starting material is thyl phenylcyanoacetate which is condensed with esters of ω-halogenated fatty cids by means of sodium ethoxide:



$$[C_6H_5-C(CN)-COOC_2H_5]\,Na + X-(CH_2)_n-COOC_2H_5 \rightarrow$$

$$C_6H_5-\overset{\overset{\displaystyle CN}{|}}{\underset{\underset{\displaystyle COOC_2H_5}{|}}{C}}-(CH_2)_n-COOC_2H_5 + NaX$$

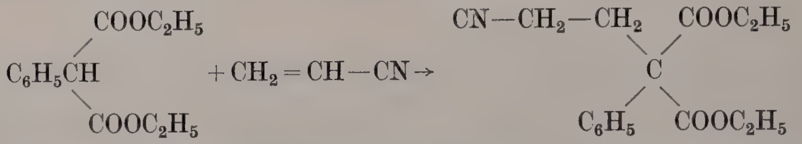
When treated with alcoholic potassium hydroxide, the reaction product is sa-onified and partially decarboxylized in one operation, thus yielding the potas-ium salt of an ω-phenyl-ω-cyanocarboxylic acid:



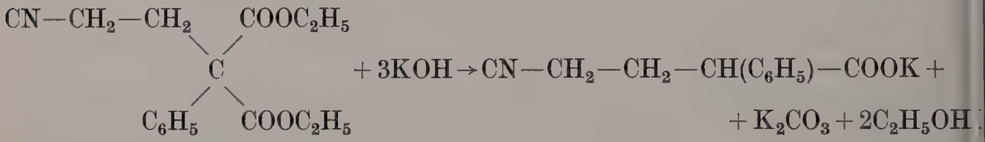
$$C_6H_5-\overset{CN}{\underset{COOC_2H_5}{C}}-(CH_2)_n-COOC_2H_5+3KOH\rightarrow C_6H_5-CH(CN)-(CH_2)_n-COOK+K_2CO_3+2C_2H_5OH$$

In this way β-phenyl-β-cyanopropionic acid and γ-phenyl-γ-cyanobutyric acid were previously prepared (2). In the present publication δ-phenyl-δ-cyanovaleric acid and ε-phenyl-ε-cyanocaproic acid are described.

When diethyl phenylmalonate is treated with acrylonitrile in the presence of a small quantity of sodium ethoxide, diethyl β-cyanoethyl-phenylmalonate is obtained



When treated with alcoholic potassium hydroxide the product is saponified and decarboxylized, thus yielding the potassium salt of α-phenyl-γ-cyanobutyric acid:

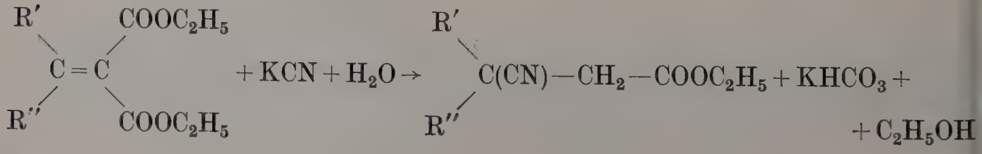


The cyano acids hitherto described have the cyano group attached to a $-CH_2$- or $-CRH$-group and are thus to be regarded as primary or secondary nitriles. The preparation of tertiary cyano acids is no problem in the case of α-cyanoacids. They are conveniently prepared by alkylation of cyanoacetic ester. Other types of tertiary cyano acids may be prepared in special cases. Thus β, β-diphenyl-β-cyanopropionic acid was prepared in good yield by SALMON-LEGAGNEUR (4) by the action of the sodium salt of diphenylacetonitrile on ethyl bromoacetate in alcoholic solution and saponification of the cyano ester obtained:

$$(C_6H_5)_2C(CN)Na+Br-CH_2COOC_2H_5\rightarrow(C_6H_5)_2C(CN)-CH_2-COOC_2H_5+NaBr$$

In the same manner β-phenyl-β-cyanobutyric acid may be prepared from the potassium salt of hydratroponitrile and ethyl bromoacetate. The reaction must be carried out in ether solution in order to avoid alcoholysis of the salt of the nitrile, as hydratroponitrile has only weak acidic properties. The yield of cyano acid is low, presumably dependent on side reactions, and most of the hydratroponitrile is recovered.

Tertiary cyano acids may also be prepared by the action of potassium cyanide on alkylidenemalonic esters in alcohol-water solution:



This method, originally due to Bredt and Kallen (5), is unfortunately of very imited applicability, as only two alkylidenemalonic esters of the required type are easily available, namely isopropylidenemalonic ester and diphenylmethylenemalonic ester. (Wideqvist (6)). The latter ester does not seem to react with potassium cyanide, presumably because of steric hindrance. Isopropylidenemalonic ester, however, reacts readily with potassium cyanide yielding ethyl β-cyanosovalerate.

Unsaturated cyano acids with the cyano group in α-position may be prepared by the action of aldehydes on cyanoacetic acid. Thus benzaldehyde gives α-cyanocinnamic acid. The isomeric β-cyanocinnamic acid was prepared in the following way: Ethyl benzalmalonate was treated with potassium cyanide in water-alcohol solution, yielding ethyl β-phenyl-β-cyanopropionate according to Bredt and Kallen (5). The cyano ester was brominated in β-position, yielding ethyl β-phenyl-β-cyano-β-bromopropionate. When the bromo ester was treated with pyridine, hydrogen bromide separated out with the production of β-cyanocinnamic ester:

$$C_6H_5-CBr(CN)-CH_2-COOC_2H_5+C_5H_5N \rightarrow$$
$$C_6H_5-C(CN)=CH-COOC_2H_5+C_5H_5N,\ HBr$$

The free cyano acid is easily obtained by saponification of the ester with an equivalent amount of alkali in alcohol.

Attempts to get β-cyanocrotonic acid in the same way were not successful. The reaction products were only indefinite, semisolid sticky masses which could not be purified.

## Experimental

### Phenylcyanoacetic acid

$$C_6H_5-CH(CN)-COOH$$

Ethyl phenylcyanoacetate, prepared according to Hessler (1), Wallingford, Jones and Homeyer (7) and Wideqvist (8), was dissolved in 2-N sodium hydroxide and kept at room temperature overnight. On acidifying the solution with hydrochloric acid, the monohydrate of phenylcyanoacetic acid separated as an oil which immediately crystallized. The crystals were filtered off with suction, washed with a little cold water and dried in the air. The acid was then recrystallized twice from benzene and once from chloroform. M. p. 74—75°.

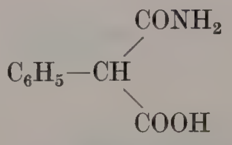
*Anal.* Subs., 0.1665 g: 9.30 ml of 0.1000-N NaOH.
» 24.87 mg: $CO_2$, 55.05 mg; $H_2O$, 11.10 mg.
Calcd. for $C_9H_7O_2N$, $H_2O$ Equ.w. 179.2; C, 60.33; H, 5.06.
Found Equ.w. 179.0; C, 60.40; H, 4.99.

A sample of the acid monohydrate was dried in vacuo over phosphorous pentoxide for twenty-four hours. The melting point after drying was 91—92°; Hessler (1) reported 92°.

*Anal.* Subs., 23.23 mg: $CO_2$, 56.99 mg; $H_2O$, 9.30 mg.
Calcd. for $C_9H_7O_2N$ C, 67.07; H, 4.38.
Found C, 66.95; H, 4.48.

Phenylcyanoacetic acid is unstable, losing carbon dioxide and being converted into benzyl cyanide even at room temperature. The acid is very rapidly decarboxylized on heating above the melting point or in water solution. The decarboxylation is strongly catalyzed by $Cu^{II}$-ions.

## Phenylmalonamic acid



A. *Phenylmalonamic ester.* Ethyl phenylcyanoacetate (5 g) was dissolved in 10 ml of conc. sulfuric acid. The solution was kept at room temperature for two hours and then poured on crushed ice, whereupon slightly yellowish crystals of ethyl phenylmalonamate separated. The ester was recrystallized from hot alcohol. The recrystallized product weighed 2.0 g and melted at 148.5°.

*Anal.* Subs., 22.29 mg: $CO_2$, 52.33 mg; $H_2O$, 12,95 mg.
Calcd. for $C_{11}H_{13}O_3N$ C, 63.73; H, 6.33.
Found C, 64.02; H, 6.50.

B. *Phenylmalonamic acid.* Phenylmalonamic ester (2.0 g) was dissolved in a hot solution of sodium hydroxide, prepared by dissolving 0.3 g of sodium in a mixture of 10 ml of methanol and 0.25 ml of water. The mixture was kept at room temperature overnight. Ether was added and the separated sodium salt was filtered off and washed with ether; yield 1.85 g. The salt was dissolved in water and the solution was acidified with dilute sulfuric acid and extracted with a mixture of ethyl acetate and ether (7: 3). On evaporating the solvent, white crystals of phenylmalonamic acid were obtained. The acid was recrystallized from hot water. It melts at 123° with evolution of carbon dioxide. The decarboxylated product solidifies immediately and melts, on further heating, at 157—158°.

*Anal.* Subs., 23.28 mg: $CO_2$, 51.89 mg; $H_2O$, 10.74 mg.
Calcd. for $C_9H_9O_3N$ C, 60.31; H, 5.07.
Found C, 60.78; H, 5.16.

A sample of phenylmalonamic acid was heated in a test tube over a small Bunsen-flame, until no more carbon dioxide was evolved. The product was recrystallized from hot water. It melted at 159°. When mixed with an authentic sample of phenylacetamide the same melting point was obtained.

### δ-Phenyl-δ-cyanovaleric acid

$$C_6H_5-CH(CN)-CH_2-CH_2-CH_2-COOH$$

A. *Diethyl α-phenyl-α-cyanoadipate.* Sodium (2.3 g) was dissolved in 37 ml of osolute alcohol. To the solution was added 19 g of ethyl phenylcyanoacetate llowed by 20 g of ethyl γ-bromobutyrate. After boiling under reflux for two ours the solution was neutral. After cooling, water was added and the sep- ated ester was extracted with carbon tetrachloride. The extract was dried ith anhydrous magnesium sulfate, the solvent was distilled off and the residue as fractionated *in vacuo*. B. p. 214—215°/10 mm. Yield 18.7 g of diethyl α- ienyl-α-cyanoadipate (62 %).

B. *δ-Phenyl-δ-cyanovaleric acid.* Thirteen grams of potassium hydroxide was ssolved in 275 ml of alcohol, and 18.7 g of diethyl α-phenyl-α-cyanoadipate as added to the solution. The mixture was boiled under reflux for three hours nd potassium carbonate separated during the boiling. The solution was then ltered and most of the alcohol was distilled off under reduced pressure through column. To the residue were added 150 ml of water and 50 ml of benzene. he benzene and 50 ml of the water were then distilled off together with the st of the alcohol. The residue was diluted with an equal volume of water, xtracted twice with ether in order to remove any unchanged ester and acidified ith 50 ml of 2-M sulfuric acid. δ-Phenyl-δ-cyanovaleric acid separated as an l and was extracted with ether. After drying with anhydrous magnesium sul- te and evaporating the solvent, the acid was obtained as an oil which solid- ied on scratching. It was recrystallized from toluene. Yield 10.4 g. The acid elts at 75—76°.

*Anal.* Subs., 26.36 mg; $CO_2$, 68,85 mg; $H_2O$, 16.13 mg.
» 0.2182 g: 9.39 ml of 0.1142-N HCl (Kjeldahl).
Calcd. for $C_{12}H_{13}O_2N$ C, 70.90; H, 6.45; N, 6.89.
Found C, 71.23; H, 6.85; N, 6.88.

### ε-Phenyl-ε-cyanocaproic acid

$$C_6H_5-CH(CN)-CH_2-CH_2-CH_2-CH_2-COOH$$

A. *Diethyl α-Phenyl-α-cyanopimelate.* Sodium (2.3 g) was dissolved in 37 ml absolute alcohol, and 19 g of ethyl phenylcyanoacetate was added to the lution followed by 21 g of ethyl δ-bromovalerate. The reaction mixture was fluxed for two hours and treated in the same manner as described for diethyl phenyl-α-cyanoadipate. B. p. 221—222°/10 mm. Yield 23.1 g (73 %).

B. *ε-Phenyl-ε-cyanocaproic acid.* Diethyl α-phenyl-α-cyanopimelate (23.1 g) was ponified by boiling for three hours with a solution of 15 g of potassium hy- oxide in 300 ml of alcohol. The precipitated potassium carbonate was filtered f, and the filtrate was treated in the same manner as described for δ-phenyl- cyanovaleric acid. ε-Phenyl-ε-cyanocaproic acid was obtained as an oil which lidified on scratching; yield 12.6 g. It was recrystallized from n-butyl ether. . p. 54—55°.

*Anal.* Subs., 25.50 mg: $CO_2$, 66.58 mg; $H_2O$, 16.06 mg.
» 0.2120 g: 8.65 ml of 0.1142-N HCl (Kjeldahl).
Calcd. for $C_{13}H_{15}O_2N$ C, 71.85; H, 6.96; N, 6.45.
Found C, 71.20; H, 7.05; N, 6.52.

## α-Phenyl-γ-cyanobutyric acid

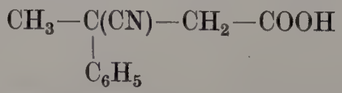
$$CN-CH_2-CH_2-CH(C_6H_5)-COOH$$

A. *Diethyl β-cyanoethyl-phenylmalonate.* Sodium (0.2 g) was dissolved in 30 ml of absolute alcohol, and 23.6 g (0.1 mole) of diethyl phenylmalonate was added followed by 7 g of acrylonitrile in two portions. The temperature of the reaction mixture raised from 18° to 38°. After cooling to room temperature, the mixture was treated with water and extracted with ether. The extract was washed with dilute sulfuric acid and dried with anhydrous magnesium sulfate. The ether was distilled off and the residue fractionated *in vacuo*. B. p. 197—198°/8 mm. Yield 20.8 g (72 %) of diethyl β-cyanoethyl-phenylmalonate.

B. *α-Phenyl-γ-cyanobutyric acid.* Twenty and eight tenths grams of diethyl β-cyanoethyl-phenylmalonate was mixed with a hot solution of 12 g of potassium hydroxide in 50 ml of alcohol. A salt separated at once. The reaction mixture was kept at room temperature for 24 hours, whereupon the salt was filtered off and washed with ether. It weighed 11.2 g and consisted mainly of potassium carbonate. The filtrate and washing were evaporated and 12 g of potassium salt was obtained. When the salt was dissolved in water and acidified with 5 ml of conc. hydrochloric acid, an oil separated, which, on shaking, solidified to white lumps of crystals. Yield 7.5 g. It was recrystallized from toluene. M. p. 89—90°.

*Anal.* Subs., 27.28 mg: $CO_2$, 70.20 mg; $H_2O$, 15.20 mg.
» 0.2055 g: 9.50 ml of 0.1142-N HCl (Kjeldahl).
Calcd. for $C_{11}H_{11}O_2N$ C, 69.80; H, 5.86; N, 7.41.
Found C, 70.17; H, 6.23; N, 7.39.

## β-Phenyl-β-cyanobutyric acid



$$CH_3-\underset{\overset{|}{C_6H_5}}{C}(CN)-CH_2-COOH$$

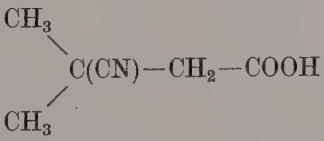
A. *Ethyl β-phenyl-β-cyanobutyrate.* In a three-necked 500-ml round-bottomed flask, immersed in an oil bath and fitted with a reflux condenser, a Hershberg stirrer and a dropping funnel, were placed 200 ml of dry isopropyl ether and 3.9 g of potassium. The oil bath was heated until the isopropyl ether boiled, whereupon the stirrer was started and the molten potassium was finely divided and evenly distributed in the ether. Hydratroponitrile (14 g, 0.1 mole.), prepared according to WIDEQVIST (9), was then added slowly through the funnel. During the addition, the potassium salt of hydratroponitrile separated as a yellow powder. When all the nitrile was converted into its potassium salt, 18 g of ethyl bromoacetate was added drop by drop. The reaction mixture was then refluxed for two hours. After cooling, the ethereal solution was treated with water, washed with dilute sulfuric acid and dried with anhydrous magnesium sulfate. The isopropyl ether was distilled off through a column and the residue was fractionated *in vacuo* by means of a Claissen apparatus. Yield 4 g of crude ethyl β-phenyl-β-cyanobutyrate, boiling at 150—180°/10 mm. The quantity was too small to permit further purification, but this proved to be unnecessary. Most of the hydratroponitrile was recovered.

B. *β-Phenyl-β-cyanobutyric acid.* Sodium (0.5 g) was dissolved in 10 ml of methanol and 0.4 ml of water was added. The solution was mixed with 4 g of crude ethyl β-phenyl-β-cyanobutyrate and a sodium salt immediately began to separate. The mixture was kept at room temperature for three days, whereupon ether was added and the salt was filtered off and washed with ether; yield 1.7 g (8 % of the theoretical, based on the original quantity of hydratroponitrile). The salt was dissolved in water and the solution was acidified with dilute sulfuric acid and extracted with ether. The extract was dried with anhydrous calcium chloride. On evaporating the ether an oil remained which, on scratching, crystallized. M. p. 80—84°.

*Anal.* Subs., 38.51 mg: 3.020 ml of 0.06725-N NaOH.
» 22.35 mg: $CO_2$, 57.46 mg; $H_2O$, 12.01 mg.
» 21.11 mg: 1.655 ml of 0.06725-N HCl.
Calcd. for $C_{11}H_{11}O_2N$ Equ.w 189.2; C, 69.80; H, 5.86; N, 7.41.
Found Equ.w 189.6; C, 70.11; H, 6.01; N, 7.39.

When kept in the air β-phenyl-β-cyanobutyric acid absorbs moisture and liquefies. After some time it solidifies again, presumably because of the formation of phenylmethylsuccinamic acid.

### β-Cyanoisovaleric acid



$$(CH_3)_2C(CN)-CH_2-COOH$$

A. *Ethyl β-cyanoisovalerate.* In a 1000-ml round-bottomed flask, fitted with a reflux condenser, was placed a solution of 40 g (0.2 mole) of diethyl isopropylidenemalonate in 500 ml of 96 per cent alcohol. A solution of 14 of potassium cyanide in 50 ml water was added and the flask was heated in an oil bath at 80° for seven hours.

The mixture was cooled under tap water, and the precipitate of potassium bicarbonate (weight 11.5 g) was filtered off with suction and washed with alcohol. The combined filtrate and wash liquor was made slightly acid with hydrochloric acid. Most of the alcohol was then distilled off through a column under reduced pressure. The residue was treated with water and extracted with benzene. The extract was washed with dilute sodium carbonate and dried over magnesium sulfate. The solvent was distilled off and the residue fractionated *in vacuo* through a Widmer column. Ethyl β-cyanoisovalerate was collected at

98°/9 mm; yield 13.2 g (43 %). An additional fraction, boiling at 144—145°/9 mm, was also obtained. It weighed 6.1 g and consisted possibly of β-cyanoisopropylmalonic ester.

B. β-*Cyanoisovaleric acid.* A solution of 2 g of sodium and 2 ml of water in 20 ml of methanol was added to 13.2 g of ethyl β-cyanoisovalerate. The mixture was kept at room temperature for 5 days. Ether was added, and the precipitated sodium salt was filtered off and washed with ether; yield 10.8 g (85 %). From the sodium salt the β-cyanoisovaleric acid was liberated by means of dilute sulfuric acid, extracted with ether and dried with calcium chloride. On evaporating the ether, the acid was obtained as white crystals. It was recrystallized from benzene. M. p. 78—79°.

*Anal.* Subs., 21.80 mg: $CO_2$, 45.49 mg; $H_2O$, 14.18 mg.
» 0.2069 g: 14.19 ml of 0.1142-N HCl (Kjeldahl).
Calcd. for $C_6H_9O_2N$ C, 56.66; H, 7.14; N, 11.02.
Found C, 56.90; H, 7.28; N, 10.97.

β-Cyanoisovaleric is easily soluble in water, ether and hot benzene. In water solution the cyano group is rapidly hydrolyzed.

## β-Cyanocinnamic acid

$$C_6H_5{-}C(CN){=}CH{-}COOH$$

A. *Ethyl* β-*phenyl*-β-*cyanopropionate* was prepared by the action of potassium cyanide on diethyl benzalmalonate according to BREDT and KALLEN (5). From 50 g of benzalmalonic ester 20 g of ethyl β-phenyl-β-cyanopropionate was obtained. B. p. 173—178°/13 mm.

B. *Ethyl* β-*cyanocinnamate.* In a 100-ml flask, fitted with a reflux condenser and a dropping funnel, were placed 17 g of ethyl β-phenyl-β-cyanopropionate and a crystal of iodine. The flask was heated in an oil bath at 95° and 15 g of bromine was added through the funnel over a period of twenty minutes. A current of dry air was then blown through the hot solution to remove hydrogen bromide and excess bromine. The flask was then cooled with tap water and 20 ml of pyridine was added carefully. A vigorous reaction took place with strong evolution of heat. The mixture was then heated to 100° for a short time. After cooling, the dark colored solution was treated with an excess of dilute sulfuric acid and the precipitated β-cyanocinnamic ester was extracted with ether. After drying and expelling the ether the reaction product was fractionated *in vacuo.* Ethyl β-cyanocinnamate was collected at 170—178°/10 mm; yield 7.5 g (44.5 %).

C. β-*Cyanocinnamic acid.* The ethyl β-cyanocinnamate (7.5 g) was mixed with a solution of 1.6 g of sodium hydroxide in 15 ml of methanol. A sodium salt separated at once as a thick paste. After two hours it was filtered off, washed with ether and dried. I was then dissolved in water and the solution was acidified with dilute sulfuric acid, whereby the β-cyanocinnamic acid separated as a white powder; yield 4 g. It was recrystallized from a mixture of ether and ligroin. The acid melts at 141.5° with dark coloring.

*Anal.* Subs., 54.50 mg: 4.678 ml of 0.06725-N NaOH.
» 25.34 mg: $CO_2$, 64.39 mg; $H_2O$, 9.84 mg.
» 21.58 mg: 1.840 ml of 0.06725-N HCl.
Calcd. for $C_{10}H_7O_2N$ Equ.w. 173.2; C, 69.34; H, 4.08; N, 8.09.
Found Equ.w. 173.2; C, 69.29; H, 4.34; N, 8.03.

## SUMMARY

A number of new mononitriles of dicarboxylic acids have been prepared by different methods: 1) By condensation of ethyl phenylcyanoacetate with esters of ω-halogenated fatty acids (δ-phenyl-δ-cyanovaleric acid, m. p. 75—76°, and ε-phenyl-ε-cyanocaproic acid, m. p. 54—55°). 2) By addition of acrylonitrile to ethyl phenylmalonate (α-phenyl-γ-cyanobutyric acid, m. p. 89—90°). 3) By condensation of hydratroponitrile with ethyl bromoacetate (β-phenyl-β-cyanobutyric acid, m. p. 80—84°). 4) By the action of potassium cyanide on ethyl isopropylidenemalonate (β-cyanoisovaleric acid, m. p. 78—79°). 5) By the elimination of hydrogen bromide from β-phenyl-β-cyano-β-bromopropionic ester by means of pyridine (β-cyanocinnamic acid, m. p. 141.5°).

An investigation of phenylcyanoacetic acid showed that this acid separates from water solution in the form of a monohydrate. It may be recrystallized from benzene and chloroform without dehydration. M. p. 74—75°. On drying *in vacuo* over $P_2O_5$, the water is given off, and the dried product melts at 91—92°.

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REFERENCES. (1) **Hessler,** Am. Chem. J., *32,* 127 (1904). — (2) **Wideqvist,** Svensk Kem. Tid., *54,* 34 (1943). — (3) —, Arkiv Kemi, Mineral. Geol., *26 A,* No. 16 (1948). — (4) **Salmon-Legagneur,** Compt. rend., *208,* 1507 (1939). — (5) **Bredt** and **Kallen,** Ann., *293,* 343 (1896). — (6) **Wideqvist,** Arkiv Kemi, Mineral. Geol., *20 B,* No. 7 (1945). — (7) **Wallingford, Jones,** and **Homeyer,** J. Am. Chem. Soc., *64,* 576 (1942). — (8) **Wideqvist,** Arkiv Kemi, Mineral. Geol., *24 B,* No. 14 (1947). — (9) —, Svensk Kem. Tid., *55,* 125 (1943).

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# Spectroscopic evidence of the participation of the cytochrome-cytochrome oxidase system in the active transport of salts

By H. Lundegårdh

With 6 figures in the text

## 1. Introduction

Extensive experimental investigations as to the relation between the aerobic respiration and the active absorption of salts in various plant organs and tissues (see 9–13, 16, 17) have shown that in the total aerobic respiration are included at least two groups of processes, 1:o the "ground respiration" which is independant of the translocation of salts and insensitive to cyanide and azide, and 2:o the "anion respiration" which is very sensitive to cyanide and directly related to the active movement of salts through the living cells. Both processes involve a complete combustion of glucose. The anion respiration is important also as a promotor of normal growth (11). The ground respiration may be characterized as a respiration at low level, maintaining life but not capable of much constructive work. Little is known about the possible interrelation of the two aerobic processes (11), but both are probably superposed on the common chain of glycolysis proceeding the end-oxidation.

The intensity of the anion respiration is quantitatively related to the intensity of absorption and transport of salts. The intensity is low in the absence of movable anions and rises immediately after increasing the supply of salts (12, 13, 16). The respiration and the absorption of salts from the medium are to the same extent controlled by low or increasing concentrations of cyanide, azide or $\alpha$, $\alpha$-dipyridyl (18) and they are also both inhibited by carbon monoxide, an inhibition which is reversed by the illumination with white light (20, Fig. 1). These facts strongly support the idea of the anion respiration being conducted by the cytochrome-cytochrome oxidase system (10). On this basis I developed a theory of the anion respiration according to which the internal transferance of electrons from the dehydrogenase system through the body of the cytochrome complex to the molecular oxygen is balanced by a transport, in the opposite direction, of free movable anions (see the scheme below).

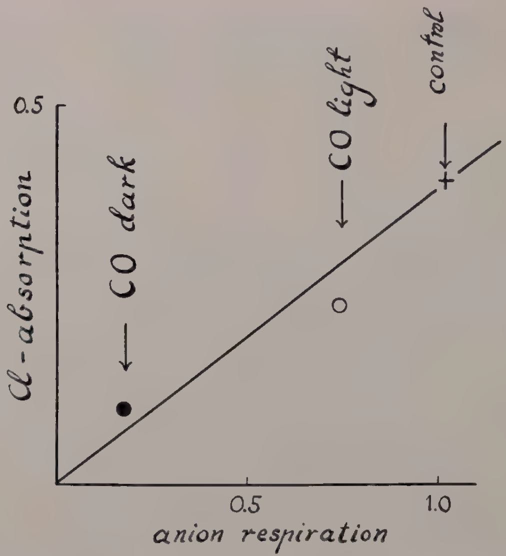
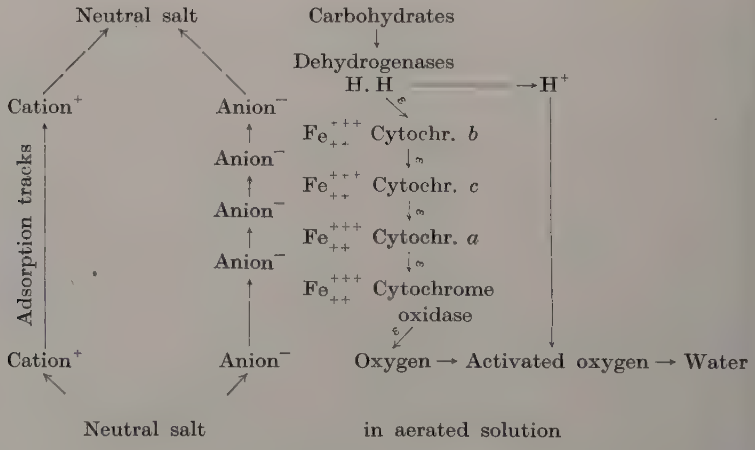


Fig. 1. The inhibition of the anion respiration and the absorption of chloride anions from KCl by carbon monoxide and the reversion of this inhibition by illumination with 14 000 Lux. After E. SUTTER (20).


Neutral salt
Cation⁺
Anion⁻
Adsorption tracks
Anion⁻
Anion⁻
Anion⁻
Cation⁺
Anion⁻
Neutral salt
Carbohydrates
Dehydrogenases
H.H
→H⁺
e
Fe⁺⁺⁺ ₊₊ Cytochr. b
e
Fe⁺⁺⁺ ₊₊ Cytochr. c
e
Fe⁺⁺⁺ ₊₊ Cytochr. a
e
Fe⁺⁺⁺ ₊₊ Cytochrome oxidase
e
Oxygen → Activated oxygen → Water
in aerated solution

If the single hemin enzymes are parts of an organized system each iron atom will at one moment release an electron and receive an anion. In the next moment it will receive one electron and release one anion, which then is catched by the next enzyme of the chain, etc. The complete system operates similarly to a micro-electrochemical element and the stream of anions outside of the enzyme body can also be figured as an electrophoresis. The equivalent movement of cations in the opposite direction is realized by the transferance of H-ions liberated at the oxidation of the dehydrogenase (simultaneously with the emission of electrons) and finally neutralized by the activated oxygen forming

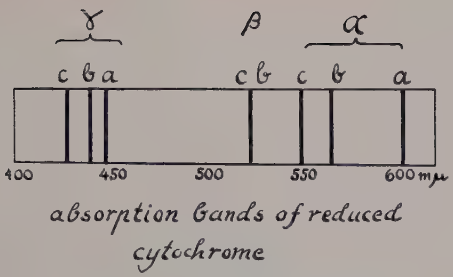


Fig. 2. Scheme of the cytochrome spectrum, showing the α-, β- and γ- groups of *a*, *b* and *c*. After KEILIN and HARTREE (6).

water. The accumulated anions are balanced by metallic cations which are transported along adsorption tracks of the protoplasmic structure.

Of the single components of the cytochrome system hitherto only cytochrome *c* has been isolated and more closely studied (21). Two more cytochromes, *a* and *b*, are frequently observed (see Fig. 2) and are obviously more firmly attached to the living structure than *c*. In some objects modifications of *b* have been observed. The cytochrome-cytochrome oxidase or cytochrome $a_3$ (6,7) is probably identical with WARBURG's "Atmungsenzym" (22). Its absorption bands in violet and orange-red are related to the bands of cytochrome *a*. The oxidase has a pronounced affinity to CN and CO and the compounds of oxidase and inhibitor show a characteristic translocation of the absorption bands, whereas the bands of *a* remain unaffected. Cytochrome *c* does not combine with CN at physiological pH-values (21, 4).

As to the cooperation of the single links in the complete cytochrome system it is commonly believed that the order is: dehydrogenase $\rightarrow b \rightarrow c \rightarrow a \rightarrow a_3 \rightarrow O_2$. The existence of intermediate links have been supposed by some authors, but according to KEILIN and HARTREE (8) all are based on insufficient evidence except that of SLATER (17) who has produced reasoned arguments in favour of an oxido-reduction catalyst linking *b* and *c*. As connecting link between the chain of glycolysis and the cytochrome system the succinic acid dehydrogenase seems to have a dominating position, but also other dehydrogenases have been under consideration, e.g. dehydrogenases of malic acid, lactic acid, and a DPH-cytochrome-reductase (1, 15), catalyzing the transferance of electrons between reduced coenzyme I and cytochrome, or a TPN-cytochrome-*c*-reductase (flavoprotein). Also in recent biochemical work the importance of a close structural linkage between the single components of the dehydrogenase-cytochrome system is emphasized (8).

For the definite statement of the identity of the anion respiration with the cytochrome-cytochrome oxidase system it has to be shown that the cytochromes are present in the studied objects and that their state of oxidation-reduction is influenced by the transport of salts. Because roots of wheat seedlings have been extensively used in this laboratory for research work on anion respiration these objects were also chosen for the spectroscopic studies. Similar results were obtained with roots of corn, however. The presence of cytochrome in plant material has been demonstrated by KEILIN (5) and Japanese scientists (23). The presence of cytochrome in yeast, acetic acid bacteria etc. is well-known. All these investigations have been made by means of visual spectroscopy and are probably, as far as higher plants concerns, uncertain. It is difficult to trace the presence of cytochromes in wheat roots by means of a hand spectroscope, because the concentration only amounts to 8–25 mg. per kg. fresh material. GODDARD, however, isolated cytochrome *c* from wheat germs and demonstrated its identity with the cytochrome from heart of cow (2, 3). Also cytochrome oxidase was found in this material.

For the present investigation a special spectroscopic technique was elaborated, by means of which the absorption spectrum of a bundle of roots, or even of single roots, can be accurately recorded and the behaviour of the absorption bands can be directly followed in the course of the experiments.

## 2. Methods

With the aid of photomultiplier cells RCA or EMI, allowing an amplification of the current from the photo-cathode amounting to $10^6$–$10^7$, a recording micro-spectrophotometer was built enabling the ligth intensity falling on the roots to be held so low that it does not at all disturb the normal functions of the tissue. A schematical sketch of the apparatus is given in Fig. 3.

As light source served a small Osram lamp (6 v. and 4.35 amp.) supplied from a large storage battery. After a start period of c. 30 minutes its emission holds very constant during several hours, a circumstance of considerable importance, if series of spectra are to be taken from one and the same object. The current is controlled by rheostates and a measuring instrument. The light from the lamp is collected by means of a large condensor lens and projected on the slit of the collimator of a Zeiss' monochromator. The outgoing beam is reflected by a prism through an objective (Zeiss' Micro-Summar 3 mm.) and finally projected on the object, which is held by an adjustable table screening off the photocell. Through a circular hole in the table the light transmitted through the object reaches the photo-cathode of the multiplier cell. The photocell is supplied in the usual way from a large anode battery and connected to a recording microvoltmeter (Speedomax, Leeds & Northrup). A shunt device enables the adjustment of the scale deviation of the instrument.

For recording a smaller or larger part of the absorption spectrum the wavelength screw of the monochromator was automatically moved in small steps by means of a toothed wheel and an electromagnetically operated hook, moving the wave-length screw one division for each point recorded on the Speedomax instrument. The period of this instrument being only 4 seconds it is thus possible to obtain a record of the whole spectrum from 650 to 380 mμ in 15–20 minutes, each point on the record representing an interval of 4 to 20 Ångström units.

In some experiments only the transparency of one line was constantly recorded, e.g. the peak of cytochrome *c* at 420 mμ (see below). In other experiments two wave-lengths were alternatingly recorded, e.g. 400 and 420 mμ representing the top and the bottom of the γ-band of cytochrome *c*. This was realized by means of a special device automatically shifting the position of the wavelength screw between the two values.

The roots were taken from seedlings cultivated in the standard manner of the institute (see 10, 12). The roots were cut to a length of 15–20 mm. and a bundle of 25–50 such pieces was introduced in a glass tube the diameter of

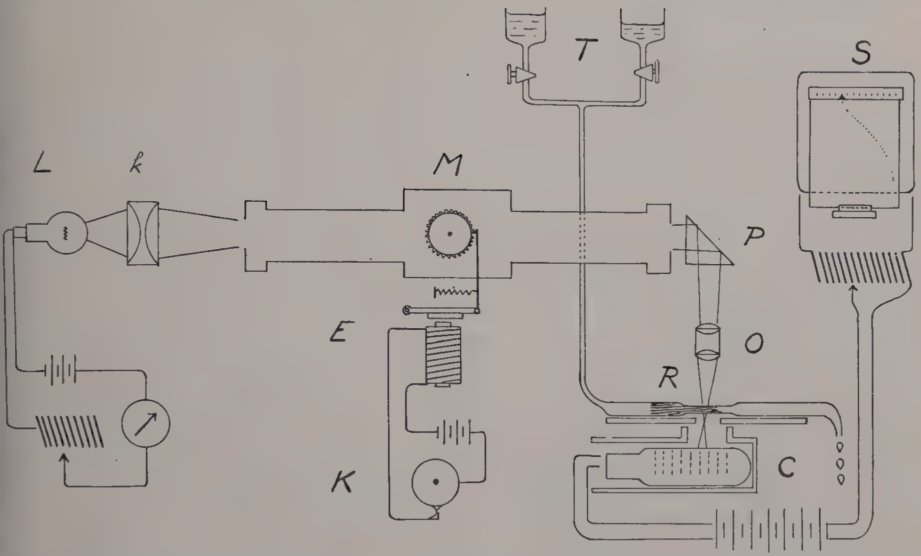


Fig. 3. Scheme of the micro-spectrophotometer. L Osram lamp, k condensor lens, M monochromator, E electromagnetic control of the wave-length screw, K contact clock for this device, P reflecting prism, O microscope objective, R recipient for the objects (bundle of wheat roots), T funnels for changing the medium around the roots, C photo-multiplier cell, S recording micro-voltmeter.

which is somewhat smaller in the middle part (see Fig. 3 R) enabling a comparatively tight packing just at the point of illumination from the monochromator. The beam measures only c. 0.1 × 2 mm. at a slit width of 0.075 to 0.10 mm. The sensitivity of the instrument is so high that a slit of 0.02–0.05 millimeter is sufficient in the middle of the spectrum (blue green) where the photo-cathode layer has its maximum of sensitivity.

The tube with the roots is connected with two funnels with solutions (water or salt solutions, inhibitors etc.) for rapid shifting over from one medium to another. The velocity of the stream can be controlled by observing the drops leaving the end of the tube.

## 3. Identification of the cytochromes

Owing to the small quantity of cytochrome present in the roots biochemical preparation is difficult. Most of the identification work in the literature has also been made spectroscopically. In the present case very accurate spectrograms were obtained and the identification of cytochromes is facilitated by the fact that three groups of bands are present in the visible region in such a way that no other known substance can be mistaken for being a hemin enzyme (fig. 2). Peroxydase is probably also present in the roots, but as far as my present experience goes it does not confuse the cytochrome spectrum.

A difficulty interfering with all quantitative measurements of absorption spectra of living objects is the fact that the reference quantity $I_0$, in colorimetric

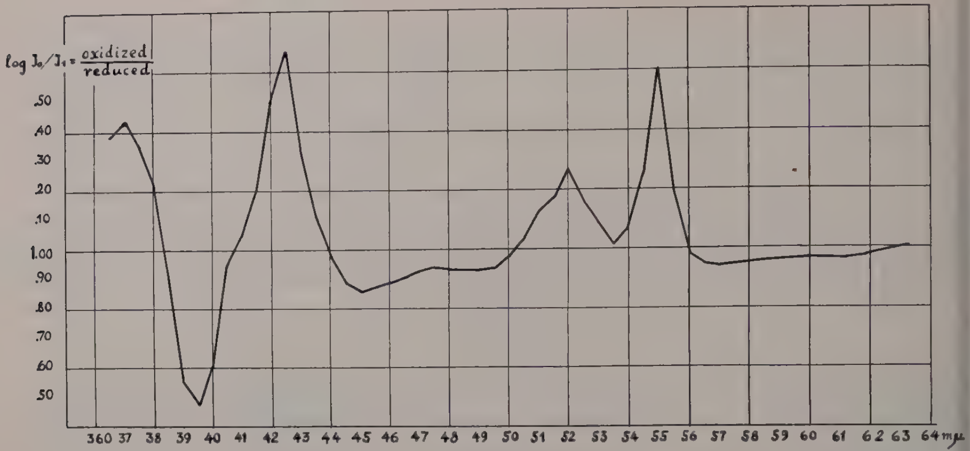
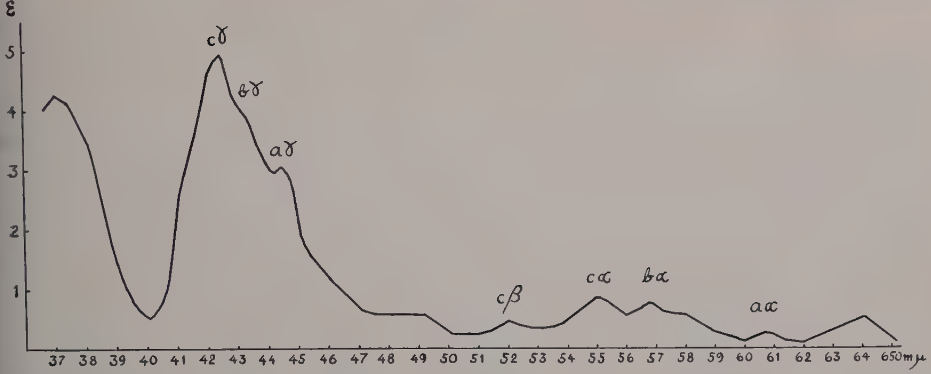


Fig. 4. The $\frac{\text{ox.}}{\text{red.}}$-spectrum of a preparation of cytochrome *c*. The concentration of the solution was 0.23 mg per 1 cc. Length of the cuvette 20 mm.

determinations representing the cuvette filled with the solute, cannot be used, because the objects cannot be made free from their coloured enzymes. Only different stages of the enzymes can be compared. In the case of cytochromes it is possible to compare the oxidized and the reduced substance, because the α- and β-bands appear only in the reduced form and the γ-bands shift position at the change from one stage to another. Under the influence of CN or CO the cytochrome oxidase is inactivated and the reducing influence of the dehydrogenases penetrates the whole cytochrome system. Roots treated with cyanide or carbon monoxide therefore show the reduced spectrum of all cytochromes. In roots treated with aerated distilled water the cytochrome oxidase still develops its oxidizing qualities. In aqua dest. the roots show the spectrum of partially oxidized cytochromes. The cytochrome spectrum of the roots was accordingly calculated as $\frac{I_0}{I_1} = \frac{\text{light absorption of roots in distilled water}}{\text{» \quad » \quad » \quad » \quad in 0.001 m. HCN}}$. The constancy of the lamp (see above) is so good that the spectrum recorded in aqua dest., followed by the spectrum recorded in HCN, can be used for the calculation of the extinction values of all wave-lengths. In the present investigation readings were taken at intervals of 5 mμ.

Only cytochrome *c* offers a quite reliable basis for identification because this enzyme was prepared in pure form (21). Through the courtesy of Professor Hugo Theorell I have been able to record the absorption spectrum of a preparation of cytochrome *c* with a purity of c. 95 %. The enzyme was dissolved in 0.01 n. HCl and aerated. According to Theorell it is then completely oxidized. After recording the ox.-spectrum the solution was neutralized and reduced with sodium hyposulphite and the red.-spectrum recorded. Calculating as above $\frac{I_0}{I_1} = \frac{\text{Oxidized cytochrome } c}{\text{reduced \quad » \quad »}}$ we get the curve shown in Fig. 4. No calculation of the molar absorption coefficient was made for these identification purposes.



ig. 5. The $\frac{\text{ox.}}{\text{red.}}$-spectrum of a bundle of 25 wheat roots at a distance from the tip of c. 12 nm. See the text. The extinction is calculated in relative values. The height of the cγ-band represents a decrease of the transparency of c. 20 %.

n other records the coefficient was calculated enabling the estimation of the pproximative concentration of cytochrome *c* in the roots.

The values for the wave-lengths of the absorption bands of the cytochromes iven in the literature are somewhat varying and, with the exception of *c* (21), robably not quite reliable, because it is very difficult to attain accurate readngs at direct visual observation and the bands have a certain width. Most onochromators (and spectroscopes) are not satisfactorily corrected. It is imortant to check the readings of an instrument at least every week by comarison with known wave-lengths. I have used the Lundegårdh flame-aggregate nd a number of known atomic lines of potassium, lithium, sodium, thallium nd strontium, scattered over the visible part of the spectrum. In my spectrocope the wave-lengths of the peak of the γ-band of cytochrome *c* were 402 x. and 412 red. resulting in a peak at 420–425 mμ in the $\frac{\text{ox.}}{\text{red.}}$-spectrum see Fig. 4). The β-and α-bands appear at 521.5 and 551.5 mμ respectively. The inimum to the left of the γ-band appears at c. 400 mμ. The transparency of he reduced cytochrome is here higher than that of the oxidized form.

The spectrum of the wheat roots, calculated as above (see Fig. 5), shows a ell developed γ-band of cytochrome *c*. Also the bands at 522 and 552 are learly recognizable but as a rule less prominent than in the pure preparation. As expected (cf. 21, 4) the γ-band of cytochrome *c* in the roots is not influenced y CN. If the roots are bathed with a solution of CO + air the γ-band is shifted somewhat towards the violet end, on an average 3 mμ.

Concerning cytochrome *b* the position of the α-band is not exactly known, ts position is given as 557–566. The spectrum of wheat roots shows a band t 570 or somewhat below which is probably *b* because also the γ-band appears s an elevation at the right side of the *c*γ-band. On an average the *b*γ-band as its peak at 435–437 mμ. KEILIN gives the value 432 mμ. Also the β-band t 530 can be recognized in many spectrograms, but a diffuse elevation of the whole green part of the spectrum in CN, perhaps due to peroxidase or some unknown compound, makes it often difficult to point out the details. Already in pure solutions of cytochrome *c* the β-band is blurred and soon disappears at high dilution.

The joint band of *a* and $a_3$ in the blue region appears as a distinct elevation. Its position varies between 445 and 455 in cyanide, on an average 450 mμ, and between 440 and 450 in CO, on an average 447 mμ as to be expected from the dislocation of the $a_3$-band with carbon monoxide. Also the *a* α-band at 600–605 mμ is visible (see Fig. 5). Its dislocation with CO has not yet been investigated. The fact must again be recalled that the spectrum reproduced in Fig. 5 is an $\frac{\text{ox}}{\text{red}}$-spectrum, the exact basis of which is still not known, as far as *a* and *b* concerns. But it may be expected that the bands show a certain displacement in comparison to the bands in the reduced spectrum, in analogy to cytochrome *c* consequently to the right in respect of the γ-bands. Further studies are required to elucidate this point.

The roots contain small quantities of flavanon (14), carotene and probably also flavin. Under aerobical conditions these substances remain unchanged after treatment with CN or CO and their spectral influence is eliminated by our method of calculating the $\frac{\text{ox}}{\text{red}}$-spectrum. Some compounds in the roots, however, are sensitive to pH and cause on the alkaline side a strong absorption beginning at c. 450 mμ and increasing towards the ultraviolet end. This end-absorption can completely screen off the violet end of the cytochrome spectrum if solutions of KCN are used (pH c. 9). The disturbance can be avoided by using KCN + HCl at pH 4–5. The pH-reaction is extremely sensitive and is developed already in the presence of small quantities of bicarbonate in the medium, but disappears again after soaking with aqua dest. containing some $CO_2$ (pH c. 5.5–6). This phenomenon must be held in mind at experiments with neutral salts, because anions are in some cases more rapidly absorbed than cations, e.g. from $KNO_3$, causing an alkalinization of the medium.

The light absorption of the reduced γ-band of cytochrome *c* in a bundle of 25–50 roots amounts to 10–20 %. As the method allows an accurate determination of differences of 0.5 % or even less the slightest change in the oxidation-reduction situation of cytochrome *c* in the living root system can be quantitatively measured. The concentration of the cytochromes is fairly constant in the whole root, a fact coinciding with my earlier statement of the uniform anion respiration at different heights of the root (12).

In twelve spectrograms with CN and eight spectrograms with CO the heights of the γ-bands were measured. As the bands of *a* and *b* appear as a somewhat diffuse elevation at the right side of the γ-band of *c*, whereas the link side of the latter remains uncomplicated, the slope of its right side was calculated from the spectrum of the pure preparation and the γ-bands of *a* and *b* taken as the additional height. If the height of *c*γ is 100 the *b*γ was 73.6 and the *a*γ 51.0 in the curves obtained after treatment with HCN. After inhibition in CO the figures were for *b*γ 79.2 and for *a*γ 64.5. Whereas *b* is practically unaffected by the type of inhibitor *a*γ is distinctly (c. 25 %) higher in CO, a fact quite in agreement with the effect of CO on the cytochrome oxidase ($a_3$) and again showing the combined effect of *a* and $a_3$.

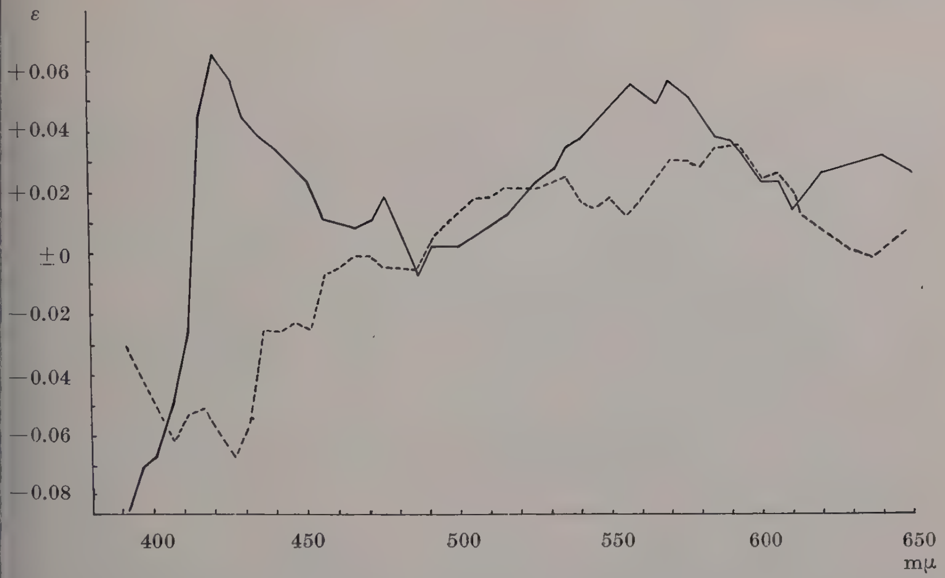


Fig. 6. Spectrograms of a bundle of corn roots. Extinction calculated from $I_0$ = the light transmission of roots in distilled water. The continuous curve illustrates the reduced cytochrome spectrum after treatment with $10^{-3}$ m. HCN. The dotted curve illustrates the decreasing reduction (or increasing oxidation) after a change of the medium from distilled water to $10^{-2}$ m. succinic acid + KOH, pH = 3.7.

As the true specific absorption coefficients of the cytochromes *a* and *b* are not known the relative quantity of them in comparison to *c* cannot be calculated from the mentioned figures. These nevertheless show that the cytochromes *a* and *b* are present in considerable quantities, in respect of *b* probably about the same as *c* (cf. 21).

## 4. The influence of neutral salts on the oxidation-reduction balance of the cytochrome system

If roots are transferred from distilled water to a solution of KCl, $KNO_3$, $Ca(NO_3)_2$ or an acid salt of an organic acid (e.g. succinic acid), the spectrum is changed in the opposite direction to that observed in cyanide, viz. the oxidation-reduction balance of the cytochromes is moved more towards the fully oxidized stage. The curve obtained with salts is consequently a reversion of the curve obtained with cyanide (Fig. 6). In some cases deviations were observed and it may be possible to find out something of the order of activation or degree of activation of the single cytochromes. This point needs further investigation. The observation of an increase of the oxidation level of the cytochromes under the influence of salts or certain organic acids is in close agreement with the theory of anion respiration, however.

## 5. The activity of the cytochrome system

According to the theory of anion respiration the transferance of electrons through the cytochrome system proceeds only slowly if few movable anions are present in the surroundings. The "density of electrons" is then low in the system, a situation obviously correlated with a partial reduction of the cytochromes. It is a well-known fact that the effectivity of the cytochrome oxidase exceeds the normal needs of the aerobic oxidation. The enzyme is "over-dimensioned," a circumstance explaining the surprisingly slow inhibition of the respiration at decreasing partial pressure of oxygen. At restricted transport of electrons, as in the case of low salt concentration, some cytochrome still remains oxydized. If now at rising availability of movable anions the "density of electrons" in the cytochrome system increases this means an increase of the oxidation power of the oxidase. As a consequence the average situation of the cytochromes is shifted over a little more to the side of oxydation. At maximum velocity of the anion respiration the oxidation power of the oxidase is considerable.

The cytochrome oxidase is extremely sensitive to cyanide. An increase of the γ-band of cytochrome *c* of 18 % was observed already in a solution of $10^{-7}$ KCN, viz. less than 0.01 mg per litre. The effect begins a few seconds after the addition of this solution and is completed within one or two minutes. A rapid reversion occurs after shifting over to distilled water.

*Table 1*

Calculated inhibition, measured as increase of reduced cytochrome c, after the application of cyanide, compared with the decrease of the anion respiration.

| KCN m/lit. | $10^{-7}$ | $10^{-6}$ | $10^{-5}$ | $3.10^{-5}$ | $10^{-3}$ |
|---|---|---|---|---|---|
| Extinction (rel.) at 425 mμ | 25 | 72 | 87 | 94 | 100 |
| Anion respiration and salt absorption[1] | 100 | —— | 57.5 | 19.2 | 0 |

The over-dimensioned power of the cytochrome oxidase (the only link in the system inhibited by cyanide) is strikingly illustrated by table 1. An anion respiration amounting to 57.5 % of the maximum value is still going on after 87 % inactivation of the cytochrome system. Only c. 25 % of the capacity of the system is needed for maintaining of an optimal anion respiration. The limiting factor near this optimum is obviously the capacity of the dehydrogenase system. Also this point will be the object of continued investigation.

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[1] See 12, tables 1 and 2.

## SUMMARY

A recording micro-spectrophotometer has been constructed by means of which he absorption spectrum of living tissue can be accurately measured. The pparatus also enables the continuous recording of one or two spectral lines.

Roots of wheat and of corn show a normal cytochrome spectrum with α-, - and γ-bands of *a*, *b* and *c*. The spectrum was calculated as the relation etween oxidized and reduced cytochrome. Roots treated with distilled water or one to several days show the enzymes partly in the oxidized form. A omplete reduction of the cytochromes appears after treatment with cyanide r carbon monoxide. The presence of cytochrome oxidase ($a_3$) is announced by slight dislocation of the combined bands of $a$ and $a_3$ under the influence f CO.

After shifting the medium from distilled water to a solution of a neutral alt (0.001–0.01 m.) or certain organic acids the oxidation-reduction balance of he cytochrome system is turned over towards the side of oxydation indicating n activation of the electron transferance in accordance with the theory of anion espiration.

The full intensity of the anion respiration is maintained at partial inhibition f the cytochrome oxydase by low concentrations of cyanide ($10^{-7}$ to $10^{-6}$ m. KCN). The time course of the inhibition and its reversal in distilled water was followed by continuous recording of the γ-band of cytochrome *c*.

REFERENCES. 1. **Bach, Dixon & Zerfas,** Biochem. Journ., 40, 1946, 229. — 2. **A. H. Brown & D. R. Goddard,** Amer. Journ. Bot., 28, 1941, 319. — 3. **D. R. Goddard,** Amer. Journ. Bot., 31, 1944, 270. — 4. **B. L. Horecker & A. Kornberg,** Journ. Biol. Chem., 165, 1946, 11. — 5. **D. Keilin,** Proc. Roy. Soc., B, 98, 1925, 312. — 6. **D. Keilin & E. F. Hartree,** Proc. Roy. Soc., B, 127, 1939, 167. — 7. ——, Bioch. Journ., 41, 1947, 500. — 8. ——, Bioch. Journ., 44, 1949, 305. — 9. **H. Lundegårdh & H. Burström,** Bioch. Zeitschr., 277, 1935, 223. — 10. **H. Lundegårdh,** Ark. f. Bot., Kungl. Vetenskapsakad., A 32, 1945, Nr. 12. — 11. ——, Lantbrukshögskolans Annal., 16, 339, 1949. — 12. ——, Lantbrukshögskolans Annal., 16, 1949, 372. — 13. ——, Physiol. Plantar., 3, 1950, 103. — 14. **H. Lundegårdh & G. Stenlid,** Ark. f. Bot., Kungl. Vetenskapsakad., A 31, 1944, Nr. 10. — 15. **V. R. Potter,** Respiratory Enzymes (H. A. Lardy), 1949, 150. — 16. **R. N. Robertson & Marjorie J. Wilkins,** Austral. Journ. Exp. Biol. Med. Sc. 23, 1948, 17. — 17. **E. C. Slater,** Bioch. Journ., 44, 1949, 305. — 18. **G. Stenlid,** Physiol. Plantar., 3, 1950, 197. — 19. **F. B. Straub,** Hoppe-Seylers Zeitschr. . physiol. Chem., 268, 1941, 227. — 20. **Erika Sutter,** Experientia, 6, 1950, 264. — 21. **H. Theorell,** Ergebn. d. Enzymforsch., 9, 1943, 231. — 22. **O. Warburg,** Schwermetalle als Wirkungsgruppen von Fermenten. Berlin 1946. — 23. **E. Yakushiji,** Acta Phytochim., 8, 1935, 325. — 24. **E. Yakushiji & K. Okunuki,** Proc. Imp. Acad. Japan, 16, 1940, 299; 17, 1941, 38.

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Vorgelegt am 11. Oktober 1950

# Zur Kenntnis der Reduktone. VII

Von Hans von Euler, Hans Hasselquist und Armin Glaser

## A. Darstellung des reinen Triose-Reduktons II

Durch das zur Darstellung des Triose-Reduktons bisher angewandte Verfahren[1] wurde ein bei etwa 140–143° schmelzendes Produkt A erhalten, mit dem wir bisher die weiteren Umsetzungen durchgeführt haben.

Bei Versuchen, dieses A-Redukton in die darin ev. vorkommenden Isomeren (Cis- und Trans-Formen, vgl. dieses Arkiv 2, No 24 S. 378) zu zerlegen wurde u. a. auch die Reaktion des Reduktons mit 1-Methoxy-butadien[2] studiert.

Wir geben einen Versuch an: 2 g Redukton A wurden mit 2,5 ml 1-Methoxy-butadien versetzt, wobei sofort eine heftige Reaktion eintrat. Nach Abkühlen und Zusatz eines gleichen Volumens Benzol fiel im Verlauf eines Tages der nicht in Reaktion getretene Teil des Reduktons aus. Er wurde abfiltriert, mit wenig Benzol gewaschen und betrug ca 900 mg Redukton. Das Filtrat lieferte beim Einengen im Vakuum bei 20° eine dunkelbraune, klebrige Masse, die sich bald in ein Gelé verwandelte. Beim Erwärmen auf dem Wasserbad mit 2 ml konc. HCl und 10 ml Alkohol bildeten sich kleine Partikeln von kautschukartiger Konsistenz. Die abfiltrierte und getrocknete Masse wog 2,7 g. Sie ist schwer löslich in den üblichen organischen Lösungsmitteln; reduziert Tillmans Reagenz nicht.

| Ausgangsmischung | |
|---|---|
| A-Redukton | 2,0 g |
| 1-Methoxy-butadien | 2,0 g |
| Summe | 4,0 g |

| Nach der Kondensation | |
|---|---|
| Triose-Redukton II | 0,9 g |
| Kautschukartige Masse | 2,7 g |
| Summe Reaktionsprodukte | 3,6 g |
| Verluste beim Einengen | 0,4 g |

Mit dem 1-Methoxy-butadien sind also 1,1 g Redukton (etwa 55 % des angewandten Präparates) in Reaktion getreten.

Das in der beschriebenen Weise gewonnene Redukton II ist gegenüber dem Ausgangsmaterial charakterisiert durch seinen höheren und schärferen Schmelzpunkt bei 153°.

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[1] Euler u. Hasselquist, Dieses Arkiv, 26 B No 5 (1948).

[2] Wir verdanken das 1-Methoxy-butadien Herrn Direktor Dr. Walter Reppe, Badische Anilin- u. Soda-Fabrik, Ludwigshafen/Rhein.

### Fraktionierung von A-Redukton mittels 1-Methoxy-butadien

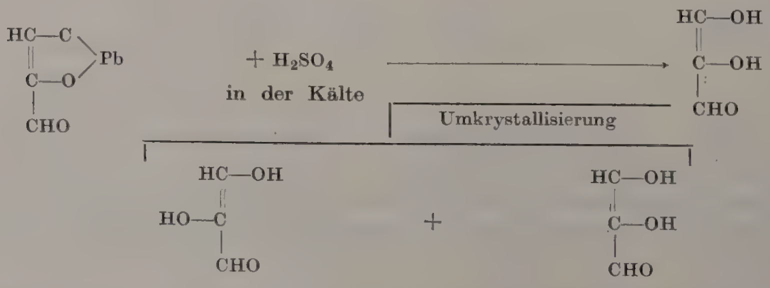
Vers. I. Versetzt man 880 mg Redukton, das aus dem Pb-Salz mit Schwefelsäure freigemacht und dann nur mit etwas Aceton gewaschen aber nicht aus Wasser umkrystallisiert worden war, mit 2,3 ml 1-Methoxy-butadien, so tritt ohne vorhergehende Erwärmung eine heftige Reaktion ein. Nach Abkühlen wurde mit der gleichen Menge Benzol verdünnt, wobei 220 mg unverändertes Redukton auskrystallisierten. Somit haben 25 % des angewandten Reduktons nicht mit dem Methoxy-butadien reagiert.

Vers. II. Wurden 880 mg Redukton I, das vorher einmal aus Wasser umkrystallisiert worden war mit 2,3 ml 1-Methoxy-butadien versetzt, so trat *ohne Anwärmung keine Reaktion ein.* Erst durch Erhitzen auf dem Wasserbad bis nahe zum Sieden setzte eine Reaktion ein, welche nun durch die Reaktionswärme die Lösung kurze Zeit im Sieden erhielt. Ein Teil des Reduktons blieb auch beim Kochen ungelöst. Nach Abkühlen wurde mit dem gleichen Volumen Benzol verdünnt; es blieben dann 500 mg Redukton (57 %) zurück, die nicht mehr mit 1-Methoxy-butadien reagierten. Dieser Reduktonanteil wurde in Aceton gelöst (er war darin schwerer löslich als das A-Redukton); die warme Lösung wurde durch Filtration von amorpher Beimischung befreit und im Vakuum eingeengt. Das Redukton fiel nun in Form kleiner harter, kugelförmiger opalescenter Krystalle aus, die im geschlossenen Rohr bei 152–153° unter Schäumen schmolzen. Das so isolierte Redukton reagiert nun mit 1-Methoxy-butadien nicht mehr.

Kontrolltitration mit TR: 3,8 mg Sbst. verbrauchten 8,8 ml 0,01–n TR
Berechnet für Redukton 8,6 ml 0,01–n TR
A-Redukton gibt das gleiche Resultat.

Es zeigte sich also: Je mehr das A-Redukton erwärmt und aus Wasser umkrystallisiert wird, um so geringer ist die Reaktion mit dem 1-Methoxy-butadien.

Unter der noch durchaus hypothetischen Annahme, dass Redukton, wenn es aus dem Pb-Salz freigesetzt wird, in der Cis-Form vorliegt, kann folgendes Schema in Betracht gezogen werden:



Transform, reagiert nicht mit 1-Methoxy-butadien. Redukton II.

Cisform, reagiert mit 1-Methoxy-butadien.

## Darstellung von Monomethyl-Redukton (HH 126)

### Kurze Einwirkung von Diazomethan

1 g Redukton II (das mit Methoxy-butadien nicht reagiert) wurde mit einem Überschuss von Diazomethan in ätherischer Lösung versetzt. Nach Beendigung der Stickstoff-Entwicklung (ca. 15 Min.) wurde die Lösung im Vakuum bei 20° auf ein kleines Volumen eingeengt. Dabei fiel der Methyläther in Form farbloser Prismen aus, welche mit etwas Äther und Methanol vorsichtig gewaschen wurden; Ausbeute 250 mg Sbst. vom Schmpt. 67–69°. Nach Umkrystallisieren aus wenig Methanol schmolz HH 126 konstant bei 70–71°.

HH 126 gibt mit $FeCl_3$ in alkoholischer Lösung starke blauviolett-Färbung, die nach etwa 1 Minute verschwindet, vermutlich wegen Hydrolyse zu Redukton. Leichtlöslich in allen üblichen organischen Lösungsmitteln ausser in Petroleumäther. An der Luft bildet sich (durch Hydrolyse?) bald Redukton sowie eine in Aceton und in Alkohol schwerlösliche farblose, amorphe Substanz. Mit Methoxy- oder Äthoxy-butadien reagiert HH 126 *nicht.*

*Analyse.*

Berechnet für $C_4H_6O_3$ $OCH_3 = 30{,}40\ \%$
Gefunden $OCH_3 = 27{,}00\ \%$

## Methylierung von Redukton II

### Längere Einwirkung von Diazomethan (im Überschuss)

Unsere früher wiederholt angestellten Versuche, die *beiden* Endiol-Hydroxyle des Reduktons zu methylieren, also den Redukton-Dimethyläther darzustellen, wurde nun mit Redukton II, und zwar mit Überschuss von Diazomethan wiederholt.

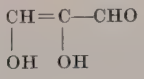
Wurden 2 g Redukton II in 10 ml Methanol gelöst und mit einem Überschuss von Diazomethan in ätherischer Lösung versetzt, so entstand sofort eine kräftige $N_2$-Entwicklung. Nach ca. 20-stündigem Stehen der Reaktionslösung wurde der Zusatz von ätherischer Diazomethanlösung wiederholt. Am nächsten Morgen wurde die Lösung im Vakuum bei Zimmertemperatur stark eingeengt und der Rückstand bei 12 mm Hg destilliert. Bei 90–91° (Badtemp. 130–140°) ging ein schwach gelb gefärbtes Öl über, das in den üblichen organischen Lösungsmitteln leicht löslich war. Sbst. HH 128. Das Öl reduziert Tillmans Reagenz *nicht.* Reagiert mit Phenylhydrazin in essigsaurer Lösung.

Das 5 Tage lang über $P_2O_5$ getrocknete Öl wurde auf seinen Methoxylgehalt analysiert.

*Analyse.*

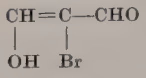
Ber. als Redukton-dimethyläther, $C_5H_8O_3$: $CH_3O = 53{,}45\ \%$
Gefunden 32,88 31,18 %

In diesem Zusammenhang erwähnen wir einige Ergebnisse über die Äthylierung der dem Redukton (I) entsprechenden Bromverbindung (II):



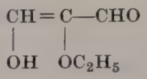
$\underset{\mathrm{OH}}{\mathrm{CH}}{=}\underset{\mathrm{OH}}{\mathrm{C}}{-}\mathrm{CHO}$

I



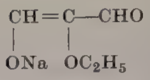
$\underset{\mathrm{OH}}{\mathrm{CH}}{=}\underset{\mathrm{Br}}{\mathrm{C}}{-}\mathrm{CHO}$

II



$\underset{\mathrm{OH}}{\mathrm{CH}}{=}\underset{\mathrm{OC_2H_5}}{\mathrm{C}}{-}\mathrm{CHO}$

III

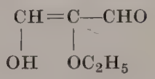


$\underset{\mathrm{ONa}}{\mathrm{CH}}{=}\underset{\mathrm{OC_2H_5}}{\mathrm{C}}{-}\mathrm{CHO}$

IV

welche M. Grard[1] bei der Untersuchung des Brom-malon-dialdehyds erhalten hat. Bei Behandlung der Verbindung (II) mit Natriumäthylat gewann er den entsprechenden Äthyläther (III).

,,La réaction s'effectue en milieu alcoolique: on détruit l'éthylate de sodium en excès par l'addition de sulfate diméthylique; après filtration de la liqueur on chasse l'alcool dans le vide à 100°, le résidu . . . met rapidement en évidence des cristaux blancs de (IV''). ,,Si l'on traite la solution aqueuse du sel de sodium par la quantité équivalente d'acide sulfurique dilué qu'on épuise par l'éther, on sépare l'aldéhyde (V)



$\underset{\mathrm{OH}}{\mathrm{CH}}{=}\underset{\mathrm{OC_2H_5}}{\mathrm{C}}{-}\mathrm{CHO}$

(V).

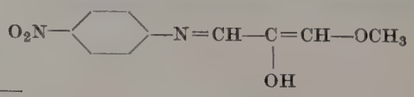
Ajoutons à solution alcoolique de cette aldéhyde, la quantité correspondante de phénylhydrazine, une coloration violette intense apparait en même temps que se manifeste un dégagement de chaleur.'' Die hierbei entstehenden Krystalle betrachtet Grard, allerdings ohne Angabe einer Analyse, unter Hinweis auf Lespieau[2] als ein Pyrazol.

In diesem Zusammenhang ist der von W. Dieckmann und L. Platz[3] isolierte Chlormalonaldehyd bemerkenswert, welcher denselben in 1-Phenyl-4-chlorpyrazol überführen konnte.

### Umsetzung der Sbst. HH 128 mit p-Nitranilin

Liess man 100 mg der Sbst. 128 mit 200 mg p-Nitranilin in 1 ml abs. Alkohol 2 Stunden reagieren so krystallisierten rote Nadeln aus, die, mit wenig Alkohol gewaschen bei 195–196° unter Zersetzung schmolzen (Sbst. A).

Aus der Mutterlauge erhielt man nach Eindunsten 60 mg kleine, *gelbe* verfilzte Nadeln, die nach Reinigung mit wenig Alkohol den Schmpt. 230–233° zeigten (Sbst. B). Durch Umkrystallisieren stieg der Schmpt. der Sbst. B (ohne Zersetzung) auf 238–239°. Mischung mit der Sbst. 119, dem Mono-methyl-äther des Redukton-p-nitranilins gab *keine Depression*, Sbst. B ist also mit dem Mono-methyl-äther des Redukton-nitranilins identisch.



(Sbst. B) (HH 119)

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[1] M. Grard, Ann. de Chim. (10. sér.) 13, 336 (1930).

[2] Vgl. M. Lespieau, C. r. Acad. Sc. Paris 133, 538 (1901).

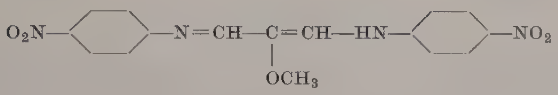
[3] W. Dieckmann u. L. Platz, Chem. Ber. 37, 4638 (1905).

Sbst. A wurde durch einmaliges Umkrystallisieren aus Alkohol in Form dunkelroter Nadeln erhalten, die unter Zersetzung bei 226–227° schmolzen. Krystallisiert man dieselben aus Eisessig um, so erhält man kleine, *gelbe* prismatische Nadeln, die beim Trocknen ihren Krystall-Eisessig verlieren und in die dunkelrote Form übergehen. Diese Sbst. HH 129, reduziert Tillmans Reagenz weder in neutraler noch in alkalischer Lösung.

Ziemlich schwer löslich in allen üblichen organischen Lösungsmitteln.

*Analyse* von HH 129.

| | | |
|---|---|---|
| Berechnet für | $C_{16}H_{14}O_5N_4$ | $CH_3O$ = 9,07 % |
| Gefunden | | = 9,08 % |



## Versuch zur Trennung der Cis- und Trans-Form von Redukton-p-Nitranilid

1,5 g Redukton in 100 ml abs. Alkohol wurde einer siedenden Lösung von 2,36 g p-Nitranilin in 50 ml abs. Alkohol zugesetzt. Nach 15 Min. Sieden begann das Reaktionsprodukt in Form von kleinen roten Nadeln auszukrystallisieren. Nach weiteren 40 Min. Sieden liess man die Lösung bei 20° 18 Stunden stehen; das sich abscheidende Rohprodukt (2,77 g) schmolz bei 232–233° unter Zersetzung.

Nach möglichst schnellem Auflösen des Rohproduktes in möglichst wenig Eisessig wurde die Lösung zur Krystallisation gestellt.

Sbst. A. Ziemlich grosse, rotviolette, metallglänzende Prismen, bei 226° unter starker Zersetzung schmelzend.

Sbst. B. Aus dem Filtrat von A schieden sich sofort Krystalle in Form von gelben Nadeln, Schmpt. 237° (Zersetzung) ab.

| Substanz A | Substanz B |
|---|---|
| 50 mg Sbst. bei 100° im Vakuum getrocknet: Kein Gewichtsverlust. | 44,4 mg Sbst. bei 100° 18 Stunden getrocknet. Gewichtsverlust = 10,3 mg; die gelbe Farbe geht dabei in rotorange über.<br>$C_9H_8O_4N_2 + CH_3COOH$<br>Ber. 22,4 %<br>Gef. 23,2 % |

Kocht man Sbst. A längere Zeit mit Eisessig so geht sie in Sbst. B über.

Löst man Sbst. A in Aceton, so erhält man eine rote Lösung. Beim 4-tägigen Stehen bei Zimmertemperatur wird sie klar gelb und an den Wänden des Gefässes setzen sich gelbe, verfilzte Nadeln ab, die beim Trocknen orangerot werden und bei 237° unter Zersetzung schmelzen. Somit tritt eine Umlagerung in die Sbst. A ein. Die gelben Krystalle können sich also aus den roten durch Anlagerung bilden.

### Methylierung der Sbst. A

200 mg Sbst. A wurden in 10 ml Methanol aufgeschlämmt und mit 40 ml einer ätherischen Lösung von Diazomethan versetzt. Nach 24-stündigem Stehen wurde im Vakuum bei 20° eingeengt. Dabei fielen kleine gelbe Prismen aus, welche, mit Äther gewaschen bei 163–165° ohne Zersetzung schmolzen. Nach Umkrystallisieren aus Aceton erhielten wir grosse farblose Prismen vom Schmpt. 169–170°. Verbraucht nicht TR.

Leicht löslich in warmem Aceton, Methanol und Äthanol; ziemlich löslich in warmem Eisessig und Äthylacetat. Wenig löslich in Äther; unlöslich in Benzol, Petroleumäther und Wasser. HH 118.

### Methylierung der Sbst. B

1,5 g Sbst. B wurden in 100 ml Methanol suspendiert und mit 400 ml einer ätherischen Lösung von Diazomethan versetzt. Nach 24-stündigem Stehen wurde im Vakuum eingedunstet, fielen orange-gelbe Nadeln aus, die, mit Äther gewaschen bei 238–239° ohne Zersetzung schmolzen. Nach Umkrystallisieren aus Aceton wurden lange orangerote Nadeln vom Schmpt. 242–244° (ohne Zersetzung) erhalten. Sbst. HH 119.

HH 119 ist ziemlich löslich in warmem Alkohol, Eisessig, Äthylacetat; wenig löslich in Äther, unlöslich in kaltem Benzol, Petroleumäther und Wasser. Reduziert Tillmans Reagenz nicht.

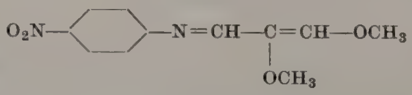
Ein besonderer Versuch zeigte, dass Sbst. 119 nicht weiter methyliert werden kann und nicht in methylierte Sbst. A übergeht.

*Analyse.*

Subst. A (HH 118)

Berechnet für $C_{11}H_{12}O_4N_2$ $CH_3O— = 26,28$ %

Gefunden 24,40; 24,20 %



Subst. B (HH 119)

Berechnet für $C_{10}H_{10}O_4N_2$ $CH_3O— = 13,97$ %

Gefunden 13,99 %

### Redukton II — p-Nitranilid + Essigsäureanhydrid (HH 127)

Hält man 370 mg Sbst. Red.-nitranilid mit 2,5 ml Essigsäureanhydrid bei gelindem Sieden, so geht das Ausgangsmaterial in Lösung. Nach 7 Min. war alles gelöst und das Reaktionsprodukt begann auszufallen; dann wurde die Lösung noch 15 Min. etwas unterhalb der Siedetemperatur gehalten. Die beim Abkühlen auf Zimmertemperatur ausfallenden Krystalle (200 mg) wurden mit wenig Eisessig gewaschen und schmolzen dann bei 230–232° unter Zersetzung. Beim Umrystallisieren aus Eisessig wurde die Sbst. HH 127 in Form langer, gelber, rismatischer Nadeln von rötlichem Metallglanz erhalten, welche unverändert ei 230–232° schmolzen. Wurde die Umkrystallisation sehr schnell durchgeführt, o *fluoreszierten* die sehr dünnen kleinen Nadeln stark mit grüner Farbe

HH 127 ist leicht löslich in Aceton, löslich in warmem Methanol, Äthanol nd Eisessig, schwer löslich in Wasser. Gibt mit $FeCl_3$ in alkoholischer Lösung eine Färbung. Die Analyse zeigt, dass eine Monoacetyl-Verbindung vorliegt.

*nalyse.*

| | |
|---|---|
| Berechnet für $C_{11}H_{10}O_5N_2$ | $N = 11{,}20$ % |
| Gefunden | $N = 11{,}12$ % |

## B. Methylierung des p-Aminobenzoesäure-Triosereduktons II

Im Triose-Redukton war, wie in der vorhergehenden Mitteilung[1] erwähnt urde, bisher nur das eine der beiden Endiol-Hydroxyle methyliert worden[2]. as andere Endiol-Hydroxyl konnte acetyliert werden, sodass ein Monomethyl-edukton-acetat entstand.[3] Schon daraus ging hervor, dass es kein sterischer influss ist, welcher die Bildung eines Dimethyläthers erschwert.

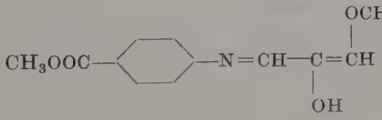
Es war nun zunächst gelungen, 2 Methylreste in *das mit p-Aminobenzoesäure erbundene* Redukton einzuführen.

500 mg p-Aminobenzoesäure-redukton wurden mit einer Ätherlösung von iazomethan versetzt und bei Zimmertemperatur 3 Tage stehen gelassen, wobei lles in Lösung ging. Aus der im Vakuum eingeengten Lösung schied sich ein l ab, welches von Krystallen durchsetzt war. Das Reaktionsgemisch wurde it Äther-Benzol versetzt und gerieben; das Öl ging dabei in Lösung, die urückgebliebenen Krystalle wurden abfiltriert[4]. Ausbeute 100 mg vom Schmpt. 66–168°. Die in Aceton, Äthylalkohol und Benzol lösliche, in Petroläther und asser unlösliche Substanz lässt sich aus Benzol mit Petroläther umfällen. Zur eiteren Reinigung wurde die Substanz HH 125 zweimal aus Alkohol umkrystallisiert, wodurch sie in Form gelber Prismen vom Schmpt. 167–168° geonnen wurde. Dieselben gaben eine schwache braunrote Färbung mit $FeCl_3$ n Wasser oder Alkohol.

*nalyse.*

| | | |
|---|---|---|
| Berechnet für $C_{12}H_{13}O_4N$ | $CH_3O = 26{,}39$ % | |
| Gefunden | 26,33 | 26,42. |

Substanz HH 125 ist demnach als p-Aminobenzoesäureester-redukton-methyläther anzuehen.



Stockholm, Universität, Inst. für organ.-chem. Forschung.

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[1] EULER u. H. HASSELQUIST, Dieses Arkiv 2 No 24 (1950).

[2] EULER u. MARTIUS, Lieb. Ann. 505, 73 (1933). — Euler u. H. Hasselquist, Dieses Arkiv.

[3] EULER u. H. HASSELQUIST, Rec. Trav. Chim. P.-B. 69 (1950).

[4] Aus dem Filtrat konnten noch weitere 50 mg von Sbst. 125 gewonnen werden, Schmpt. 60–164°. Eine dritte Krystallisation ergab eine sehr geringe Menge eines Produktes vom chmpt. 130—155°.

Tryckt den 18 januari 1951

Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 10

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# Einfluss von Triose-Redukton und Derivaten desselben auf Gärung und Wachstum von Hefen

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Mit 8 Figuren im Text

Der Umstand, dass Triose-Redukton — es soll hier der Kürze halber einfach als "Redukton" bezeichnet werden — zu vermutlichen Zwischenprodukten der alkoholischen Gärung in enger Beziehung steht, hat die Erwägung nahegelegt, dass das Redukton selbst in einer Gärungsphase auftritt. Sollte dies zutreffen, so müssten zugesetzte Redukton-Mengen vergärbar sein. Die diesbezügliche experimentelle Aufklärung schien von um so grösserem Interesse, als ja das viel diskutierte Schema des Verlaufes der alkoholischen Gärung noch keineswegs sichergestellt ist.

Redukton kommt, wie auch Askorbinsäure, als Wirkstoff des Stoffwechsels von Mikroorganismen in Betracht. Man könnte erwarten, dass die von O'MEARA und COCKER[1] für Bakterien gemachten Angaben und Annahmen auch bezgl. Hefen gelten. Was allerdings mit den hier an einer Oberhefe und einer Torula angestellten Vorversuchen[2] schwer vereinbar schien.

Dass bei der Vergärung von Glukose durch Oberhefe, und zwar bei variierendem pH eine Entfärbung des Tillmans-Reagenzes (TR) eintritt wurde schon früher erwähnt[3]. Als im Anschluss an diese Beobachtungen dann der Einfluss des zu einem Gärsystem zugesetzten Reduktons auf die Gärungsgeschwindigkeit (ccm $CO_2$ per Stunde) untersucht wurde zeigte sich bei Verwendung einer frischen Oberhefe bei Hp 6,3 die Gärungsgeschwindigkeit durch 1% Redukton bis etwa auf die Hälfte der bei dieser Acidität[4] normalen vermindert; dabei verschwand ein Teil des Reduktons. Die Versuche, die im Folgenden mitgeteilt werden, betreffen den Einfluss von Redukton und Reduktonderivaten auf den Eintritt und die Induktionszeit der Gärung einiger Hefen und sollen nur zeigen, dass zugesetztes Redukton auch bei Anwendung gärkräftiger Hefen nicht als Aktivator sondern als Hemmungsstoff wirkt. In welcher Form oder Bindung Triose-Redukton oder ein anderes, TR-reduzierendes Redukton in der gärenden Hefe vorkommt ist noch nicht bekannt, lässt sich aber vielleicht auf Grund der hier im Gang befindlichen Untersuchung an Bakterien beurteilen.

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[1] R. A. Q. O'MEARA ET AL., Nature, 154, 796 (1944). — W.COCKER, R. A. Q. O'MEARA ET AL., Biochem. J. 45, 373 1949.

[2] EULER u. H. HASSELQUIST, Experientia (Karrer-Festschr.) 5, 81 (1949).

[3] EULER u. E. KLUSSMAN, Dieses Arkiv, 11 B, No. 12 (1933).

[4] Diese für die Hefe nicht natürliche Acidität wurde gewählt in Rücksicht auf die gleichzeitige Untersuchung der Reduktionswirkung gegen TR.

## Experimentelles

Zur Untersuchung kamen folgende Hefen:

1) *Saccharomyces cerevisiae*, var. *ellipsoideus* (HANSEN) DEKKER, und
2) *Saccharomyces carlsbergensis*, HANSEN, beide aus Delfter Stammkulturen im Institut für Lebensmittelchemie gezüchtet.

Die Gärlösungen, in welchen sich die Hefen bei allen Versuchen befanden, waren folgendermaassen hergestellt:

Verdünnter Malzextrakt wurde mit HCl auf pH 4,4 eingestellt, bei 1 Atm. Überdruck 15 Minuten lang autoklaviert und der gebildete Niederschlag durch Filtrieren entfernt.

Dieses Substrat ist in DURHAM-Röhren bzw. Penicillin-Kolben mit automatischer Pipette geteilt und sterilisiert worden. Zu 4 Raum-Tln Substrat das 6,37% reduzierenden Zucker enthielt, wurde 1 Raum-Tl der das Redukton oder Redukton-Derivat enthaltenden sterilisierten Lösung oder (in den Kontrollversuchen) 1 Raum-Tl dest. Wasser gegeben.

Gleichzeitig wurden auch Parallelserien angestellt mit Röhren bzw. Kolben in denen Malzextrakt und Präparat-Lösungen zusammen autoklaviert wurden.

Redukton und Redukton-Sulfanilsäure waren in dest. Wasser gelöst, das p-Aminosalicylsäure- (PAS)-Redukton in 1/20 n. Natronlauge. Jede Präparat-Lösung wurde mit Bromphenol-blau als Indikator auf pH = 4,7 eingestellt; dies war auch die Endacidität der filtrierten Gärlösung.

Impfung wurde bei Gärungslösung mit einer dicken Hefesuspension, bei Vermehrungsversuchen mit sehr schwachen Suspensionen gleichzeitig und mit gleichen Mengen ausgefürt.

Die $CO_2$-Entwicklung wurde bei den in Fig 1 bis 6 angegebenen Versuchen in DURHAM-Röhren bestimmt. Die Versuchspunkte, welche den angegebenen Kurven zugrunde liegen, sind Mittelwerte aus je 2 bzw. 4 Versuchen. Die mit Quecksilber kalibrierten inneren Röhren wurden nach der $CO_2$ Entwicklung mit einem Masstab in Millimetern abgelesen.

Auf die geringe Genauigkeit dieser Bestimmungen wurde schon hingewiesen. Jedenfalls zeigen die Versuche deutlich die Verzögerung des Gärungsbeginnes (die Verlängerung der Induktionszeit) durch die zugesetzten Präparate und die Abhängigkeit dieses Einflusses von ihrer Konzentration.

Der Einfluss der zugesetzten Substanzen auf die Gärgeschwindigkeit (ccm $CO_2$ per Stunde) konnte in den primitiven DURHAM-Röhren nicht genau bestimmt werden, besonders im Falle von *Sacch. ellipsoideus* wo die Vergärungzeit viel kürzer war als bei *Sacch. carlsbergensis.* (9 — bzw. 24-Stunden.)

Vermehrung von Hefen haben wir durch gebildete Hefe-trockensubstanz-Bestimmungen am dritten Tage untersucht mit Hilfe von 1, G, 4, Jenaer-Glasfiltern, nach Durchwaschen mit dest. Wasser, bzw. 1/20 n. Natronlauge im Falle von P. A. S.-Redukton.

Alle Versuchs-Serien sind bei +28°C im Thermostaten inkubiert worden.

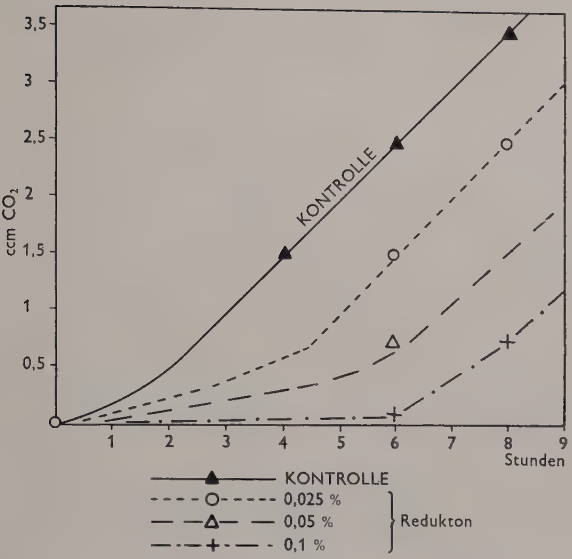

Fig. 1. *Saccharomyces cerevisiae* var. *ellipsoideus* HANSEN.

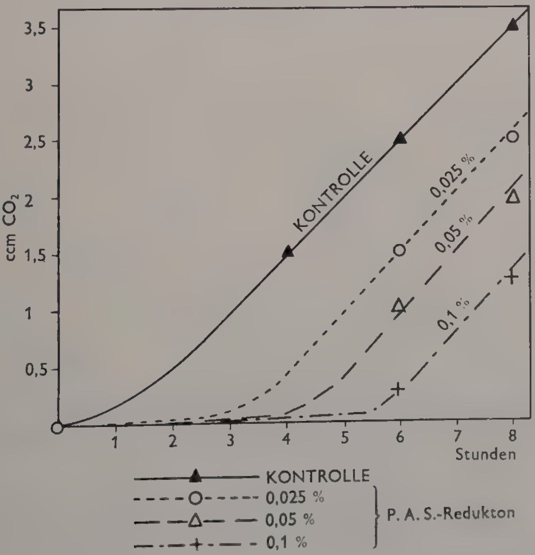

Fig. 2. *Saccharomyces cerevisiae* var. *ellipsoideus* HANSEN.

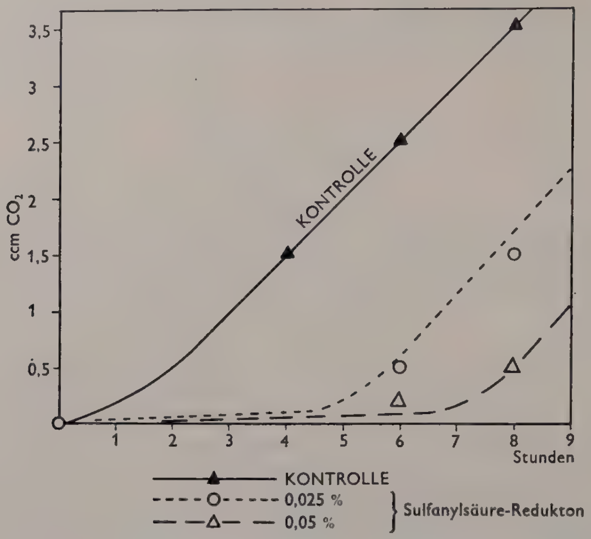

Fig. 3. *Saccharomyces cerevisiae* var. *ellipsoideus* HANSEN.

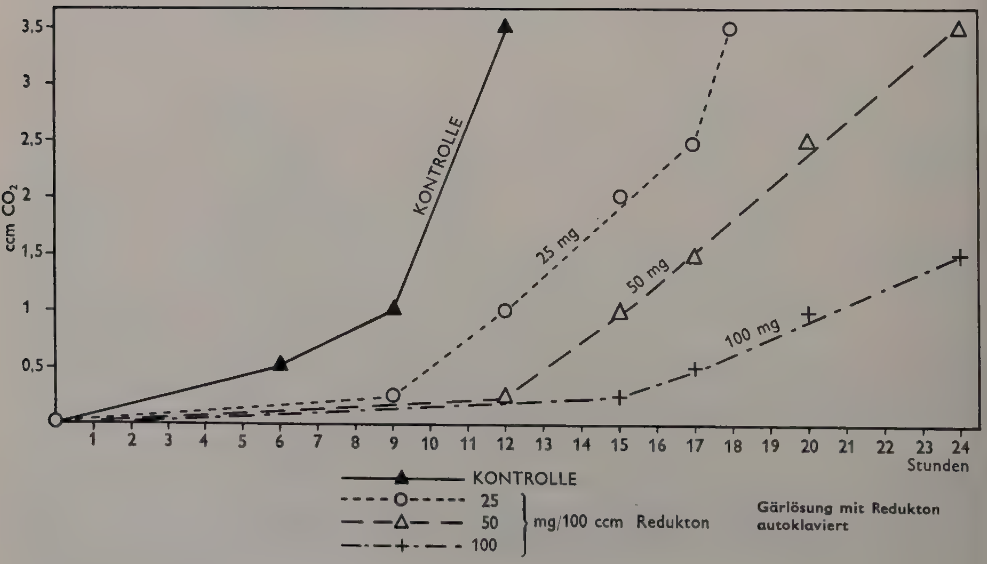

Fig. 4. *Saccharomyces carlsbergensis* HANSEN.

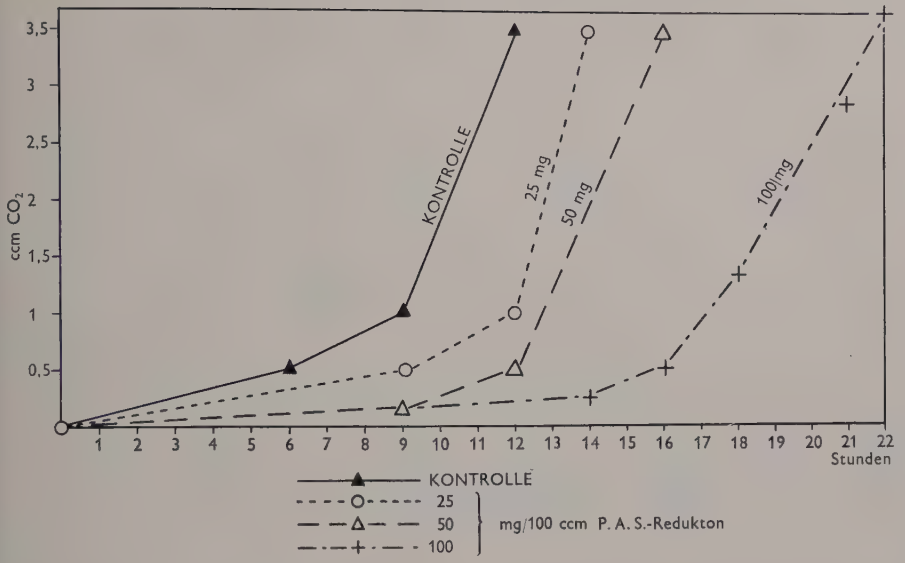

Fig. 5. *Saccharomyces carlsbergensis* HANSEN.

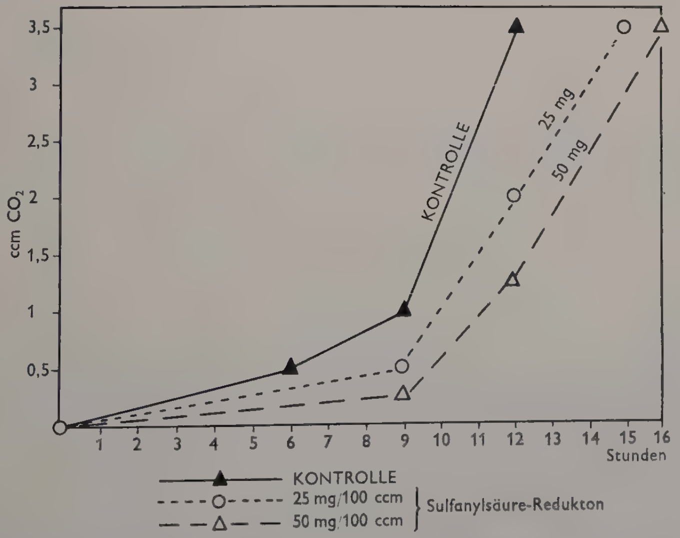

Fig. 6. *Saccharomyces carlsbergensis* HANSEN.

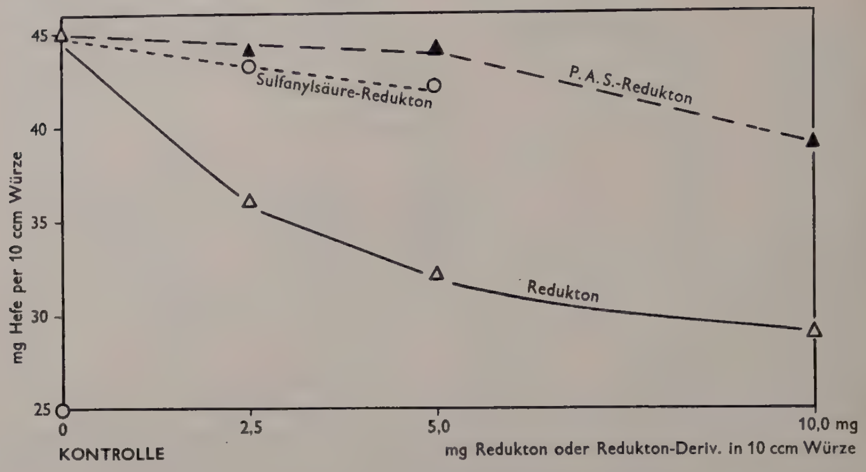

Fig. 7. *Saccharomyces cerevisiae* var. *ellipsoideus.*

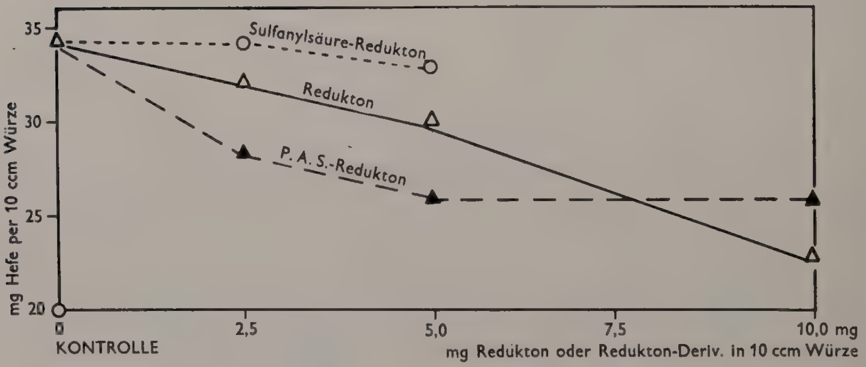

Fig. 8. *Saccharomyces carlsbergensis.*

## Resultate

Die Ergebnisse der Gärungsversuche und der Wachstumsversuche sind für die beiden angewandten Hefen in 8 Fig. zusammengestellt. Wie bereits betont, können wir den mit *Saccharomyces ellipsoideus* ausgeführten Versuchen nur den Einfluss des Reduktons bzw. seiner Derivate auf die Anfangsgeschwindigkeit der Gärung bzw. Inkubationszeit der Hefe entnehmen.

Aus den wesentlich länger (24 Std.) fortgesetzten Versuchen mit *Saccharomyces carlsbergensis* lassen sich bereits Anhaltspunkte über die Gärungsgeschwindigkeit bei den verschiedenen Zusätzen gewinnen. Bei beiden Hefen macht sich schon durch Zusätze von 25 mg/100 ccm eine deutliche *Hemmung* geltend. Dieselbe nimmt mit steigender Konzentration zu. Sie ist auffallenderweise bei Redukton am grössten, bei Sulfanylsäure-Redukton am geringsten.

Auch hinsichtlich der Wachstumshemmung, gemessen durch Zunahme der Hefe-Trockensubstanz, wird, wie aus den Fig. 7 u. 8 hervorgeht, in den angewandten Versuchszeiten der grösste Effekt durch Redukton erreicht.

Triose-Redukton verhält sich, wie ein Vergleich mit früheren Ergebnissen ergibt, ganz anders als Ascorbinsäure.

Es lässt sich noch nicht entscheiden, ob die hier mitgeteilten experimentellen Ergebnisse die Annahme zulassen, dass in der Hefe, wie in Bakterien eine enge Beziehung zwischen dem Wachstum und der Bildung von Triose-Redukton besteht. Will man die bemerkenswerten Gesichtspunkte von O'MEARA und COCKER beibehalten und auf Hefen übertragen, so kann man annehmen, dass allgemein in Mikroorganismen eine als spezifischer Wachstumsstoff funktionierende, hochaktive Verbindung des Reduktons — etwa mit einem Eiweissbestandteil, einer Aminosäure oder einem Polypeptid vorkommt. Mit der Isolierung eines solchen, von einem Redukton sich ableitenden Wachstumsstoffes ist unser Arbeitskreis beschäftigt.

Wir danken Herrn Dipl.-Ing. P. ROJOWSKY für seine Mitarbeit bei den Versuchen.

Stockholm, Institut für Lebensmittel-Chemie der Techn. Hochschule und Vitamin-Institut der Universität.

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# On accumulation of inorganic pyrophosphate in the cleaving sea urchin egg caused by lithium ions.

By Per Eric Lindahl and Karl-Heinz Kiessling

When Herbst in 1892 discovered the peculiar effect of Li-ions upon the sea-urchin development, he was aware that the essential feature of the morphological modifications caused by $Li^+$ is a displacement of the boundaries between the presumptive primitive organs in the direction of the animal pole. In this way the $Li^+$-treated larvae acquire a more vegetal appearance than normal ones, and are accordingly characterized as "vegetalized". As is well known, vegetalization is combined with a more or less complete exogastrulation. This will not be considered here, but will be discussed in a comprehensive paper in the near future.

Runnström (1928 a, footnote p. 261) measuring the respiration of the developing $Li^+$-treated sea-urchin egg (stage of development not indicated) observed an inhibition which he considered to be caused by a decreased dispersion of the protoplasmic colloids. By studying the effect of a series of $Li^+$-concentrations on the respiration of the cleaving sea-urchin egg, Lindahl (1933, 1934) found that $Li^+$ only exerts an inhibition upon the rise of respiration. The latter had on kinetic basis been suggested by Gray (1927) to be composed of two parts, one remaining constant and the other increasing exponentially. Lindahl stated, further, that the inhibition being related only to the rising part of the respiration follows the law of mass action. The same was established when studying the action of $Li^+$ on the degradation of glucose in bakers' yeast. Just as the effect of $Li^+$ on the morphogenesis in the sea-urchin larvae is counteracted by $K^+$ (Runnström, 1928 b), the inhibition of respiration in the sea-urchin egg and of the fermentation and respiration of glucose in yeast-cells were found to be diminished by $K^+$. Lindahl (1933, 1934) suggested that $Li^+$ exerts an inhibiting effect on similar chemical reactions in the two cases, and thus probably inhibits also in the sea urchin egg a (oxydative) degradation of carbohydrates. From these facts and the general parallelism between respiratory and morphological susceptibility (Lindahl, 1933, 1936), which may occasionally be obscured by a comparatively low oxygen-permeability (Lindahl, 1936, Lindahl and Öhman, 1938) Lindahl (1935, 1936) joining the gradient hypothesis of Runnström concluded that in the sea-urchin egg the animal gradient (animal morphogenetic principle, Lindahl, 1941) is probably related to carbohydrate degradation.

Further arguments in favour of this hypothesis were gained *in vitro* experiments. The methylene blue reduction with hexosemonophosphate as hydrogen donator and a centrifuged egg-extract as enzyme material is inhibited by $Li^+$

(LINDAHL, 1936). The quantitative relationship between $Li^+$-concentration and inhibition follows also here the law of mass action as was the case in similar experiments with pyocyanin as a mediator and oxygen as an electron acceptor (LINDAHL and ÖHMAN, 1938).

Determinations of the R. Q. shortly after fertilization and in the blastula stage show a rise in R. Q. (ÖHMAN, 1940). This means that the rising fraction of respiration has a higher R. Q. than the one forming at least 85% of the respiration shortly after fertilization. If the latter fraction is considered to remain constant until the beginning of the migration of primary mesenchyme, a calculation of R. Q. of the increasing fraction gives a value near unity. As pointed out above this very fraction is inhibited by $Li^+$.

Experiments in which isolated animal halves were treated with different substances related to carbohydrate metabolism, have given additional support to the above mentioned hypothesis. As well known there exists in the sea-urchin egg a tendency of extension of the animal differentiations, which in the intact egg is restrained by the vegetal material. When this is removed, the animal half acquires a more animal character than in the normal egg. If those metabolic processes which form the bases of the determination of animal diferentiations should be promoted by introducing compounds normally metabolized in these processes, an increase in the extension of the animal differentiations should be expected. Indeed, HÖRSTADIUS and STRÖMBERG (1940) obtained an animalization compared with the control by treating the animal halves with sodium pyruvat. The same result was obtained by HÖRSTADIUS and GUSTAFSSON (1947) using propandiol phosphate (cf. LINDBERG, 1945, 1946) phosphogluconic acid and lactate. — Also in intact eggs an animalization has been caused by treatment with pyruvate (AROSIO, CITTERIO, RANZI and TOSI, 1949).

If the animal morphogenetic principle in the developing sea-urchin egg is related to carbohydrate degradation, compounds known as inhibitors of carbohydrate metabolism in varying organisms and tissues would be expected to cause vegetalization. Along this line of thought several authors have performed experiments. Thus RUNNSTRÖM (1935), treating unfertilized *Arbacia* eggs with iodoacetate, obtained larvae with or without poorly developed oral arms and stomodaeum, and concluded: "Hence it seems probable that a carbohydrate breakdown is a necessary condition in the morphological differentiation of the anterior, or "animal" part of the larva". However, neither NEEDHAM and NEEDHAM (1940) nor LINDAHL (1940) nor TAMINI (1943) were able to observe any vegetalization caused by monoiodoacetate or its amide. LINDAHL pointed out that the defects appearing in the oral field and the oral arms rather depend upon an inhibition of differentiation than upon a change in determination, and emphasized the much less specificity of the inhibition of respiration caused by iodoacetate than that caused by $Li^+$. However, the failure of monoiodoacetate to produce vegetalization is now more easily understandable, since it was shown by LINDBERG and ERNSTER (1948) that the oxydation of glucose by homogenized sea-urchin eggs in the presence of diphosphopyridine nucleotide and pyocyanin is not inhibited by this compound.

Anyone of several conditions as a lack of specificity, inability of penetrating into the interior of the cells or the formation of slightly soluble compounds with ions of sea water (cf. LINDAHL, 1940) may lead to the failure to cause vegetalization of phloridzine (NEEDHAM and NEEDHAM, 1940; LINDAHL, 1940;

TAMINI, 1943), dl-glyceric aldehyd (LINDAHL, 1939, 1940; NEEDHAM and NEEDHAM, 1940, TAMINI, 1943), and NaF (LINDAHL, 1940; TAMINI, 1943).

In 1944 the action of $Li^+$ on the aerobic glucose degradation in bakers' yeast was reinvestigated and confirmed by the senior author (unpublished). At the same time the influence of $Li^+$ upon the phosphate metabolism was studied. Hydrolysis-curves revealed the accumulation under the influence of $Li^+$ of a fraction which was totally hydrolyzed in seven minutes by N $H_2SO_4$ at 100° C. without being identical with adenosine triphosphate. This fraction was first considered to be an ester but was identified as inorganic pyrophosphate upon isolation (LINDAHL and LINDBERG, 1946).

The last-related findings on yeast cells provide a further possibility to compare the metabolism of the dividing sea-urchin egg with the oxydative degradation of glucose in the yeast-cell. We have therefore taken advantage of our stay at the zoological station at Naples (February—March 1950) to investigate whether $Li^+$ also causes an accumulation of inorganic pyrophosphate in the sea-urchin egg or not.

## Experimental.

In the 4 experiments carried out, the eggs of the sea-urchin *Paracentrotus lividus* were used. The eggs were removed from the ovaries in the usual way and washed in a slow hand-driven centrifuge. Immediately after fertilization 40 ml. of the egg suspension were transferred into each of 2 rectangular troughs (24×17×5 cm.) containing 540 ml. of sea-water. To the control culture 60 ml. of sea water were added, to the $Li^+$-culture 60 ml. of isotonic (0.65 M.) LiCl solution[1]. The concentration of $Li^+$ was thus 0.065 M. The troughs were closed with glass-plates and placed on a shaking-machine (cf. LINDAHL, 1936) and gently shaken for 8.5 to 10.5 hours at 15—16° C. At the end of the cultivation, the control being just about to hatch, or having just hatched, some tenths of a ml. of suspension were removed from the troughs, the eggs washed three times in a small hand-driven centrifuge, and cultivated further in petridishes to make possible the control of the quality of the eggs used and the estimate of the degree of vegetalization promoted by the $Li^+$-treatment. These cultures were inspected about 2 days after fertilization.

The eggs of the suspensions in the troughs were centrifuged down in the large hand-driven centrifuge, and the volume brought to 15—16 ml. To fix the eggs 20% of trichloracetic acid was added in an amount to make the final concentration 4%. The suspension was then congealed and stirred in this state until only minute egg fragments could be observed at microscopic inspection. After thawing and raising the temperature of the sample to that of the laboratory, 1.0 ml. was measured off and transferred to a combustion tube for Kjeldahl analysis.

The volume of the rest of the sample was measured, cooled to 0° C. and centrifuged until the supernatant was clear. From the latter a certain amount (10—13 ml.) was pipetted off in a 25 ml. measuring-flask and brought as nearly as possible to pH 7.0 with test-paper. The flask was filled up with distilled water and the sample stored for analyses at 0° C.

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[1] The LiCl solution was prepared by dissolving $Li_2CO_3$ precipitated twice from hot water in N HCl until pH 7.0 was reached. The solution was then titrated with respect to Cl and diluted.

Pyrophosphate was analysed according to COHN and KOLTHOFF (1942), the pyrophosphate contents of the samples being obtained as differences between the value of a blanc with a known amount of added pyrophosphate and the corresponding values of the samples with the same amount of added pyrophosphate. To 20.0 ml. of the sample transferred to a 400 ml. Fernbach flask, 2.0 ml. c. 0.15 M. pyrophosphate solution, 5.0 ml. 0.2 M. ortophosphate solution, 1.0 ml. 2.0 M. $CaCl_2$ solution, and 5.0 ml. acetate buffer solution of pH 6.3 were added. The pyrophosphate was then precipitated by the addition of 17.0 ml. 0.4 M. $CdAc_2$, and the mixture was shaken over night. The coarse precipitate was filtered off on filter paper (Munktell 1 F), washed there with distilled water until free from orthophosphate, and dissolved with 30% $H_2SO_4$. The solution was transferred into a 500 ml. measuring flask and filled up; 1.0 ml. was taken for hydrolysis and orthophosphate was analysed according to MARTLAND and ROBINSON (1926). In the blancs the sample was exchanged for the same amount of distilled water.

Because of the relatively high phosphate value of the blancs the error of the determinations of the sample pyrophosphate is rather great. Thus amounts the error of values of pyrophosphate P of the size of 1.3—1.4 mg. per 25 ml. sample (cf. Table 1) to 8%, and of 0.5 mg. pyrophosphate P to 20 %.

The Kjeldahl N was determined according to PARNAS (1938).

In all experiments the control cultures gave normal plutei. The $Li^+$-treated cultures will be characterized by using a similar classification as that utilized by LINDAHL and ÖHMAN (1938), the series of vegetalized types — a "pluteus shaped larva", a "larva with a terminal oral field", a "larva lacking a concave oral field (strongly reduced ectoderm)" — being arranged as to increasing vegetalization.

Experiment 18/3: A few pluteus-shaped larvae; the remaining ones with terminal oral fields.

Experiment 19/3: A small number of larvae with terminal oral fields; the remaining lacking concave oral fields.

Experiment 20/3: Culture lost.

Experiment 21/3 About 30 % pluteus-shaped larvae, 60 % larvae with terminal oral fields, and 10 % larvae lacking concave oral fields.

Obviously, in all experiments the $Li^+$-treatment has caused a marked vegetalization.

The pyrophosphate analyses (Table 1) show that an accumulation of pyrophosphate is caused by $Li^+$ also in the developing sea-urchin egg. When the three $Li^+$-cultures from March 18, 19, and 21 are seriated according to their degree of vegetalization as well as according to their amount of pyrophosphate per unit nitrogen found in the embryos, the same order is obtained.

## Discussion.

The repeatedly demonstrated occurence also in animal tissues of inorganic pyrophosphate (CORI, 1942; CROSS, TAGGART, COVO, and GREEN, 1949; HELLER, KARPIAK, and ZUBIKOVA, 1950; the present observations) and of inorganic pyrophosphatase (LOHMAN, 1928; KAY, 1928; ROCHE, 1931; MINNITI, 1939; NAGANNA

*Table 1*

| Date | mg. N / ml. fixed susp. | mg. pyrophosp. P / 25.0. ml. sample | mg. pyrophosp. P / ml. fixed susp. | mg. pyrophosp. P / mg. N |
|---|---|---|---|---|
| 18.3 control | 5.44 | $< 0.1$ | $< 1 \cdot 10^{-2}$ | $< 0.2 \cdot 10^{-2}$ |
| 19.3 » | 5.82 | 0.5 | $5 \cdot 10^{-2}$ | $0.8 \cdot 10^{-2}$ |
| 20.3 » | 5.35 | $< 0.1$ | $< 1 \cdot 10^{-2}$ | $< 0.2 \cdot 10^{-2}$ |
| 21.3 » | [1] | $< 0.1$ | $< 1 \cdot 10^{-2}$ | $< 0.2 \cdot 10^{-2}$ |
| | | | | Mean: $< 0.2 \cdot 10^{-2}$ |
| 18.3 $Li^+$ | 6.12 | 1.4 | $12 \cdot 10^{-2}$ | $2.0 \cdot 10^{-2}$ |
| 19.3 » | 5.64 | 1.4 | $15 \cdot 10^{-2}$ | $2.7 \cdot 10^{-2}$ |
| 20.3 » | 5.42 | 1.3 | $12 \cdot 10^{-2}$ | $2.2 \cdot 10^{-2}$ |
| 21.3 » | 6.39 | 1.3 | $10 \cdot 10^{-2}$ | $1.6 \cdot 10^{-2}$ |
| | | | | Mean: $2.13 \cdot 10^{-2}$ |

[1] This Kjeldahl sample was lost. As the pyrophosphate content was found to be very low in this culture, the exact N-value, being of the same order of magnitude as in the other experiments, is of little importance.

and Menon, 1948; Cross, Taggart, Covo, and Green, 1949; Gordon, 1949, 1950) supports the idea that inorganic pyrophosphate may be looked upon as a metabolite in animal cells.

The accumulation of inorganic pyrophosphate caused by $Li^+$ may be effected either by $Li^+$ increasing the rate of formation or decreasing the rate of conversion of inorganic pyrophosphate. The only enzyme known to catalyze reactions involving pyrophosphate groups was discovered in yeast-preparations by Kornberg (1948). It catalyzes the reversible reaction

$$\left\{\begin{array}{l}\text{diphosphopyridine nucleotide} + H_2P_2O_7 \rightleftarrows \\ \rightleftarrows \text{adenosine triphosphate} + \text{nicotinamide mononucleotide.}\end{array}\right.$$

However, too little is known about the significance of this reaction to base a discussion of the relevant problem upon it.

In experiments by Cori and Ochoa (Cori, 1942) inorganic pyrophosphate appeared in rat-liver preparations after aerobic oxidation of glutamate, pyruvate, or succinate. Cross, Taggart, Covo, and Green demonstrated in the oxydasing cyclophorase system a formation of inorganic pyrophosphate which is interpreted as originating by decomposition from the first phosphorylation product, from which phosphate is said to be transferred to hexose. In this transfer two steps have been recognized: 1) from the primary ester to adenylic acid, 2) from adenosine triphosphate to hexose. Significant of the two cases reported above is the lack of a phosphate acceptor (glucose, fructose, or mannose) as a condition for the formation of inorganic pyrophosphate. Supposing that $Li^+$ acts in a similar system in the living cell, the nearest assumption would be that it blocks the transfer of phosphate from the first phosphorylation product to hexose.

The accumulation of inorganic pyrophosphate caused by $Li^+$ inhibiting a pyrophosphatase is less probable, as this enzyme, independent of its origin (bakers' yeast, liver, electrical tissue, erythrocytes, brain), is activated by $Mg^{++}$ and inhibited by $Ca^{++}$ and other bivalent ions (cf. Bailey, 1942, Bailey and Webb, 1944; Naganna and Menon, 1948; Gordon, 1949, 1950). Unpublished experiments by the senior author on yeast-cells demonstrate a competition between $Li^+$ and $K^+$ for some group in the affected enzyme when respiration is inhibited by $Li^+$. Also hexokinase (EULER and ADLER, 1935; OHLMEYER, 1937) and phosphofructokinase (TAYLOR, 1947) which are engaged in the transfer of phosphate from adenosine triphosphate to glucose and fructose-6-phosphate respectively require magnesium or manganese ions for activity, and are thereby presumably out of discussion. However, BOYER, LARDY, and PHILLIPS (1943) found that potassium ions, in addition to manganese or magnesium ions, increase the transfer of phosphate from 3-phosphoglycerate to creatine by accelerating the phosphate transfer from 2-phosphopyruvate to the adenylic acid system.

As to the attack of $Li^+$ when causing vegetalization, it has been convincingly shown that $Li^+$ promotes changes in the colloidal state of the protoplasm, or some of its constituents, towards a coarsening or aggregation of particles (cf. RUNNSTRÖM, 1928; LINDAHL, 1936; RANZI, 1947 a, b; AROSIO, CITTERIO, RANZI, and TOSI; RANZI, 1950) and that, besides, it reacts with an enzyme causing thereby disturbances in the carbohydrate metabolism to which the determination of animal differentiations seems to be chemically or energetically coupled (cf. p. 97). However, the "phase specificity" of high $Li^+$ concentrations in causing vegetalization (LINDAHL, 1940) seems to indicate that the action upon the metabolism is the *primary* attack although the action upon the protoplasmic structure strengthens the realization of the primary effect, as do other treatments coarsening the protoplasmic colloids. This will be further discussed in a comprehensive paper.

We are very much indebted to Professor R. DOHRN and the laboratory staff of the Zoological station at Naples for facilitating our work. Thanks are also due to Mr. L. KARLSSON for carrying out the pyrophosphate analyses. This investigation has been supported by grants from the Swedish National Science Research Council, O. E. NYCANDER'S research fund and "Längmanska Kulturfonden".

## SUMMARY.

It has been shown that an addition of lithium ions to a culture of sea-urchin eggs (*Paracentrotus lividus*) causes an accumulation of inorganic pyrophosphate during cleavage, i.e. the period during which $Li^+$ promotes vegetalization.

LITERAURE CITED. **Arosio, R., Citterio, P., Ranzi, M. E. S. & Tosi, L.**, Rendiconti *82*, 143, 1949. — **Bailey, K.**, Biochem. J., *36*, 121, 1942. — **Bailey, K. & Webb, E. C.**, Biochem. J., *38*, 394, 1944. — **Boyer, P. D., Lardy, H. A. & Phillips, P. H.**, I. Biol. chem., *149*, 529, 1943. — **Cohn, G. & Kolthoff, I. M.**, Indust. Enginer. Chem., 886, 1942. — **Cori, C, F.**, in Symp. on respir. enzymes, Madison 1942. — **Cross, R. J., Taggart, J. V., Covo, G. A. & Green, D. E.**, J. Biol. chem., *177*, 655, 1949. — **Euler, H. v. & Adler, E.**, Hoppe Seylers Z., *235*, 122, 1935. — **Gordon, J. J.**, Nature, *164*, 579, 1949. — **Gray, J.**, Proc. Cambr. Philos. Soc., *1*, 255, 1925. — **Heller, J., Karpiak, St. & Zubikowa, I.**, Nature, *166*, 188, 1950. — **Herbst, C.**, Arch. Entw.-mech., *55*, 446, 1892. — **Hörstadius, S. & Gustafsson, T.**, Symp. Soc. Exper. biol., *2*, 50, 1948. — **Hörstadius, S. & Strömberg, S.**, Arch. Entw.-mech., *140*, 409, 1940. — **Kay, H. D.**, Biochem. J., *22*, 1446, 1928. — **Kornberg, A.**, J. Biol. chem., *176*, 1475, 1948. — **Lindahl, P. E.**, Arch. Entw.-mech., *128*, 661, 1933. — ——, Naturwissenschaften, *22*, 105, 1934. — ——, Arkiv zool., *28 B*, Nr 4, 1935. — ——, Acta zool. (Stockholm), *17*, 179, 1936. — ——, Z. Vergl. Physiol., *27*, 233, 1939. — ——, Arch. Entw.-mech., *140*, 168, 1940. — ——, Naturwissenschaften, *29*, 673, 1941. — **Lindahl, P. E., & Lindberg, O.**, Nature, *157*, 335, 1946. — **Lindahl, P. E. & Öhman, L.-O.**, Biol. Zentralblatt, *58*, 179, 1938. — **Lindberg, O.**, Arkiv kemi, *21 B*, Nr 3, 1945. — ——, Arkiv kemi, *23 A* Nr 2, 1946. — **Lindberg, O. & Ernster, L.**, Biochim. et Biophys. Acta, *2*, 471, 1948. — **Lohmann, K.**, Biochem. Z., *203*, 172, 1928. — **Martland, M. & Robinson, R.,** Biochem. J., *20*, 847, 1926. — **Minniti, G.,** Biochim. Terap. sper., *26*, 417, 1939. — **Naganna, B. & Menon, V. K. N.,** J. Biol. chem., *174*, 501, 1948. — **Needham, J. & Needham, D. M.,** J. Exper. biol., *17*, 147, 1940. — **Ohlmeyer, P.,** Naturw., *25*, 253, 1937. — **Parnas, J. H.,** Z. Analyt. chem., *114*, 263, 1938. — **Ranzi, S.,** Boll. Soc. ital. Biol. sper., *23*, 206, 1947 a. — ——, Nature, *160*, 715, 1947 b. — ——, Abstracts of XVIII Internat. Physiol. Congr., p. 403, 1950. — **Roche, J.,** *25*, 1724, 1931. — **Runnström, J.,** Protoplasma, *5*, 201, 1928 a. — ——, Acta zool. (Stockholm), *9*, 365, 1928 b. — ——, Biol. Bull., *68*, 378, 1935. — **Tamini, E.,** Rendiconti, *76*, 363, 1942-43. — **Taylor, J. F.,** Federation Proc., *6*, 246, 1947. — **Öhman, L.-O.,** Arkiv zool., *32 A*, Nr 15, 1940.

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# Sugar beet yellows virus

## Some electron microscopical observations

By H. LEYON

With 4 figures in the text

Much work has been done on the disease of sugar beet yellows virus but so far there has been no description of the virus itself.[1] A preliminary report will be given here concerning a structure which is assumed, as a hypothesis for further work, to be the virus of sugar beet yellows.

This year an investigation was started in this institute on the proteins of the sugar beet plant with special regard to its virus diseases. During studies with the electron microscope on sap from beet leaves, filaments were occasionally found in the preparations. They occurred in sap from healthy leaves but seemed to be more common in preparations from plants infected with sugar beet yellows virus. Since ciliated bacteria were found occasionally in both these preparations, all the filaments were at first supposed to be bacterial flagella. After examination of the precipitate from sap of leaves infected with yellows, and specific antiserum against such sap, more interest was paid to the filaments. Four such precipitates were studied. If the floccules were prepared for electron microscopy immediately after they had been formed, they all gave electron micrographs showing mats of entangled threads (Fig. 1), which could not of course be found in the serum.

New examinations of sap from infected beet leaves gave more detailed information. Filaments could be found in all layers of crude centrifuged sap and also in the sediments. In preparations from the top layer of the supernates more filaments were found than in any other part of the sample. Thus the filaments seem to have a lower specific gravity than the solution. Contaminated filaments could be seen in preparations from crude sap which had not been much diluted but they were found isolated in more diluted preparations (Fig. 2).

These filaments are obviously not bacterial flagella. They do not look as stiff as the cilia and do not appear together with bacteria. They are very often associated with the disk-like structures derived from crushed chloroplasts and can also be seen inside whole chloroplasts (Fig. 2, 3 and 4). Thus they seem to be formed in the chloroplasts. In preparations from crude sap they vary much in length, from about 1 μ downwards. This variation may be due to fragmentation.

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[1] According to a personal communication, rodlike structures were found last summer at Rothamsted Exp. Station, England, in a sample of unfrozen sap from beet leaves infected with yellows.

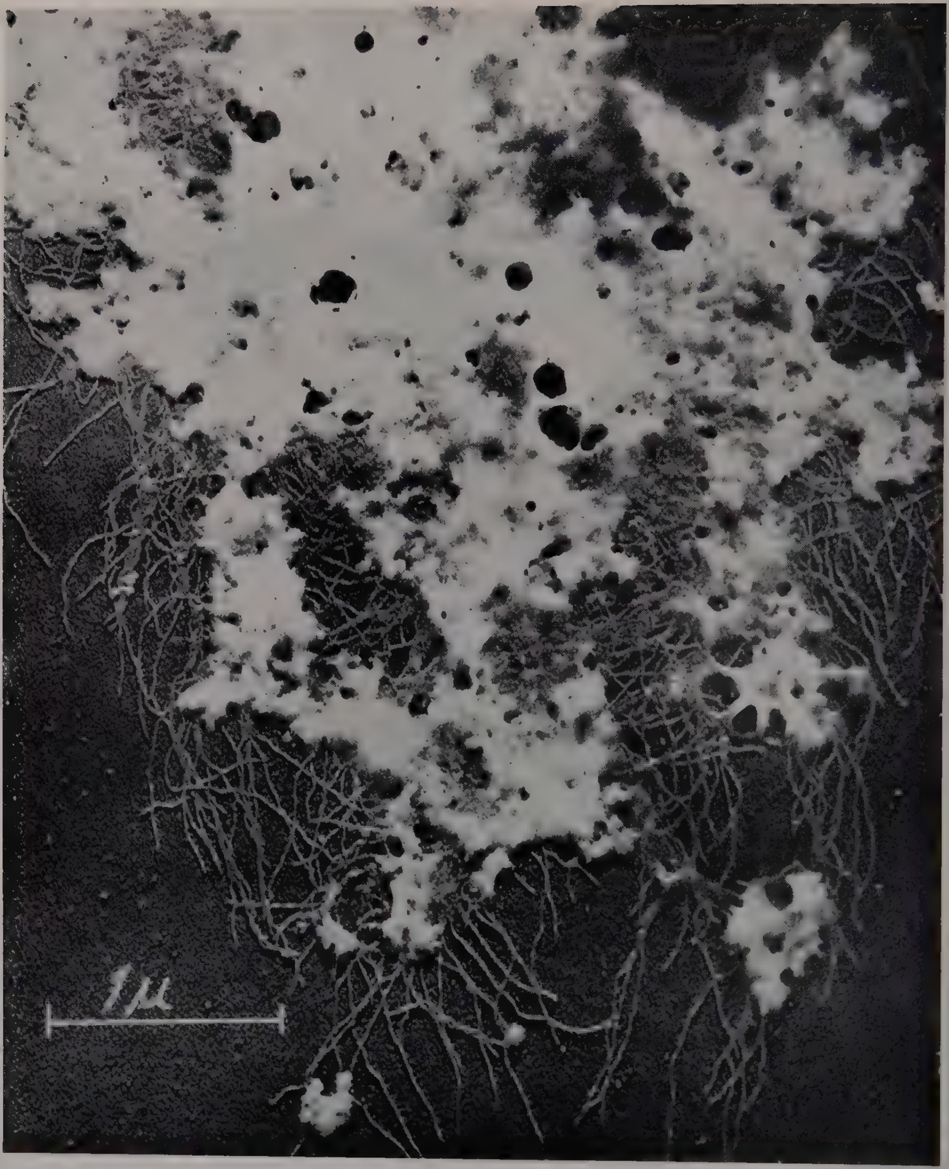

Fig. 1. Precipitate from leaves of *Chenopodium foliosum* infected with sugar beet yellows virus, and antiserum, 32000 ×.

The filaments appear with a thickness of about 10 mμ. Probably adsorbed material make them look thicker than they are. The threads of the antiserum-precipitate look somewhat thinner. In the electron micrographs the filaments seem to consist of smaller units but that might be an artefact. Filaments of the kind described were never found in preparations from healthy plants.

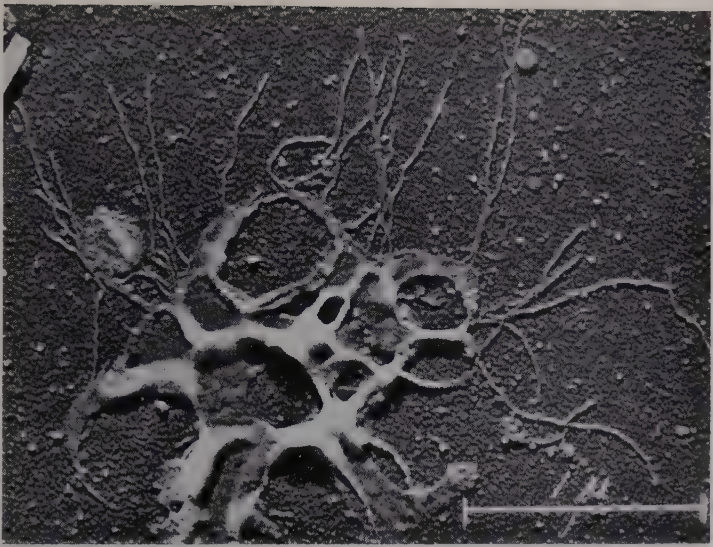

Fig. 2. Filamentous structures from beet leaves infected with yellows virus, 32000 ×.

With *Chenopodium foliosum* the same results were obtained as with beet plants. Filaments of the kind described were found in preparations from infected leaves but not from healthy ones. Sap from infected leaves of *Chenopodium foliosum* gave a precipitate with the antiserum used for the beet leaves. This precipitate was shown in the electron micrographs to consist of masses of entangled filaments (Fig. 1).

*Chenopodium foliosum* was tried, among other plants, as a host for sugar beet yellows virus. It showed severe symptoms including vein clearing followed by necrosis of the leaves. The infected leaves used in this experiment were young with marked vein clearing. The plants were equal in age to the beet plants used and had been infected at the same time. — Of the plants tried as hosts for the sugar beet yellows virus, several belonging to the family Chenopodiaceae and one species belonging to an other family (Aizoaceae) showed symptoms after infection by aphids. The results of these experiments will be given in detail in a later report.

Of course there is no complete proof so far that either of the two structures observed is the virus. Further work will be done here to settle this question. The occurrence of filaments of this sort in sap from infected leaves of two different species but not in sap from the corresponding healthy leaves and the filamentous structure of both yellows-antiserum precipitates seem, however, to be reason enough to assume — as a hypothesis for further work — that the filaments described are the sugar beet yellows virus.

**Material and methods.** The beet plant used for this investigation was *Beta Rijkmaker* which is supposed to be a cross between sugar beet and mangold. Probably the same results as here reported for *Beta Rijkmaker* and *Chenopodium foliosum* can be obtained with other infected plants but so far only these two have been studied here. The *Rijkmaker* beet

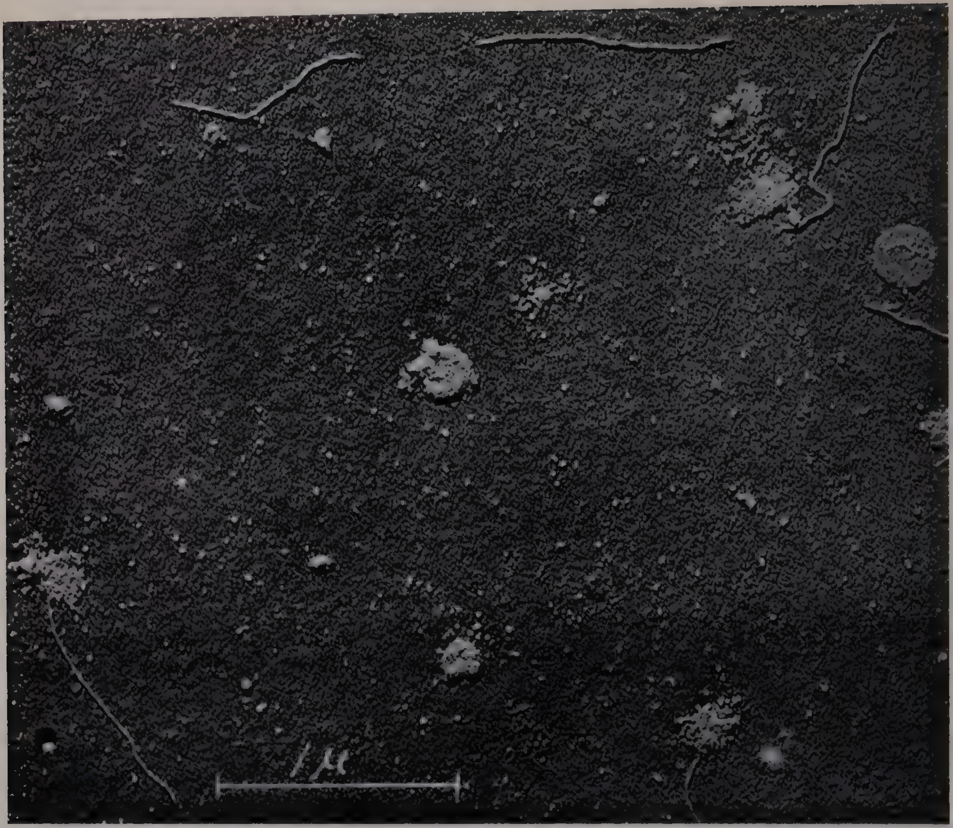

Fig. 3. Filaments and fragments of chloroplasts from beet leaves infected with yellows virus, 32000 ×.

is known from work in the Laboratorium voor Bloemenbollenonderzoek at Lisse to give strong yellows-antigen after infection. The beet plants used here were 9 weeks old with 8 leaves. They had been grown in a glasshouse and were infected 40 days before use by Myzus persicae with a swedish strain of sugar beet yellows picked up in the field.

The detached leaves were washed in water and were ground in a mortar. The sap obtained after pressing through muslin was centrifuged, usually for one hour at 3500 r.p.m. at room temperature, and then used immediately. Tenfold dilutions for electron microscopy were made with distilled water. Three infected plants and an equal number of healthy ones were studied. The antiserum had been prepared by injecting sap from infected young sugar beet plants intraperitoneally into rabbits. The serum was saturated with sap from healthy plants before use as described by BOOIJ et al. (1). Controls containing more such sap gave no reaction. The precipitate obtained with sap from infected plants was centrifuged down and prepared for electron microscopy immediately after it appeared in the mixture of sap and antiserum.

I wish to express my sincere thanks to Dr J. BLENCOWE, Rothamsted, who prepared the antiserum used, to Professor K. BJÖRLING, Ultuna, Uppsala, who

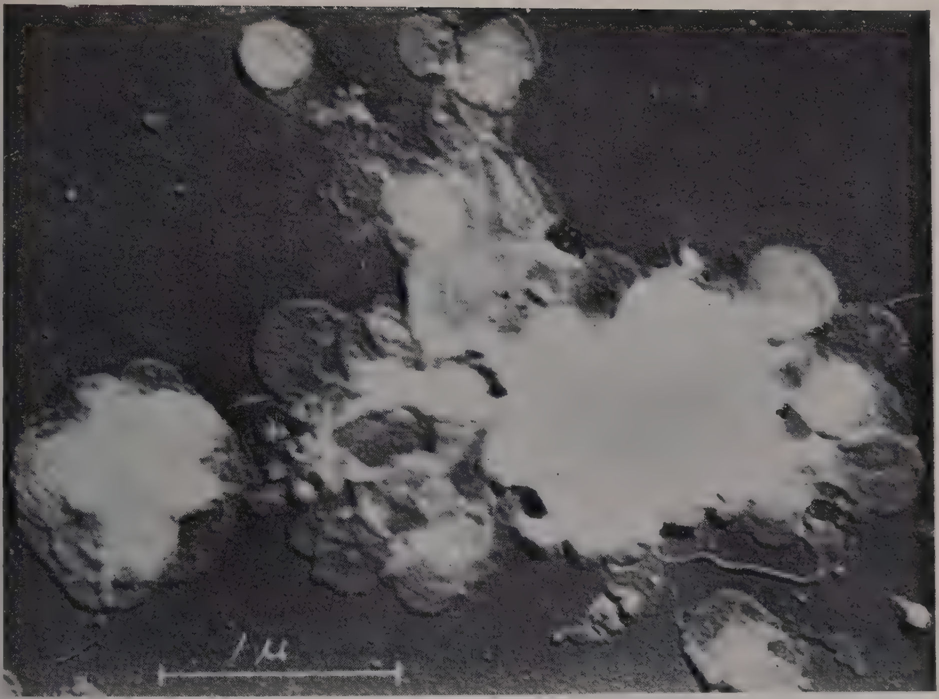

Fig. 4. Parts of chloroplasts from *Chenopodium foliosum* infected with sugar beet yellows virus, 32000 ×.

supplied the yellows strain and to Miss M. DE BRUIJN OUBOTER, Lisse, who furnished me with seeds of the "*Rijkmakers*".

The investigation during this year on the proteins of sugar beet plants were made possible by a grant from the Knut and Alice Wallenberg Foundation.

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REFERENCE: **Booij, H. L., de Bruijn Ouboter, M. P., Cremer, M. C., van Slogteren, E.:** Verslag van het Serologische Onderzoek betreffende de Vergelingsziekte der Suikerbieten 1942–1943, Mededeelingen van het Instituut voor Rationeele Suikerproductie te Bergen op Zoom, 14 (1944).

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# Studies on experimental rheumatism. I

## Attempts to produce arthritic symptoms and to stimulate autoantibody production in rats by a combined treatment

By Hans von Euler and Leo Heller

With technical assistance of Rogne Ander

Any definitive progress in our understanding of the basic pathogenetical mechanism of rheumatic diseases is rendered extremely difficult if not altogether impossible by the lack of a suitable animal model, required to display spontaneously arising or experimentally evoked symptoms of human rheumatic fever or rheumatoid arthritis. The clinical and pathological findings in animals treated by any of the available methods for the experimental production of rheumatism were in neither of the reported cases sufficiently extensive nor specific for warranting an identification with the well-known nosological entity of the human rheumatic fever. Thus the opinion expressed in 1936 by Vacirca (1), stating that "rheumatic fever has yet to be duplicated in experimental animals", remains so far unchallenged.

Among the great number of experimental techniques tried by hundreds of workers (for a review c. g. Waksman (2)) we can, however, discern a few which appear to yield somehow promising results. To that group belong 1-o: the anaphylactic methods, introduced by Cole as early as in 1904, most extensively studied by Klinge and his school (3) and, more recently, by Rich and Gregory (4), 2-o: the auto-immunization techniques of Cavelti (5), Bauer (6) and Schultz and Rose (7), based upon the hypothesis of an autoendogenous allergy (8) in rheumatism, and, 3-o: the hormone overdosage (9) and specific nutritional deficiency (10, 11, 12) techniques, elaborated in an attempt to change the normal metabolic reactivity of the organism into the specific hyperergy of rheumatic fever, in the former case according to the 'alarm' and 'adaptation' reaction scheme of Selye (13).

Whereas none of the above mentioned methods alone can be stated to yield in a consistent and reproducible way clinical and pathological syndromes undistinguishable from those of human rheumatic fever, the symptoms and histopathological lesions, evoked by them in a certain percentage — not in all — of the treated animals, seem to bear some resemblance to those of the human disease which they were intended to duplicate.

Seeking after an adequate experimental model for an attempted biochemical investigation of the enzymic activity pattern in rheumatism, we have initially tried the serum hypersensitiveness technique of RICH and GREGORY (4). Those experiments met with a partial success (14) only. The necessity of intravenously injecting rabbits with highly 'unphysiological' massive doses of foreign protein, the paucity and relative unspecificity of the pathological lesions produced, the lack of the distinct, we may say dramatical, clinical background, characteristic for human rheumatic fever, and finally the appearing of joint swelling only after a rather drastic treatment : direct intraarticular injection of antigen into the joint of the sensitized animal — the whole cannot be considered as a reproduction of the natural disease and makes the experimental serum hypersensitivity of the rabbit rather inadequate as a model of human rheumatic fever.

Our next step was to try a combined treatment, consisting of a simultaneous application of several of the above mentioned methods. We assumed that the true pathogenetical mechanism of rheumatic fever may represent a result of an interaction of several factors, having only a slight rheumatogenic effect when acting separately but being highly effective when synchronized with one another.

The so far negative results of such a combined treatment, which will be reported below, do not, in our opinion, finally disprove the above hypothesis, which we consider worth further trials, using different combinations of known slightly rheumatogenic factors. Our view receives apparent support in the promising results of a desoxycorticosterone acetate treatment, combined with chronic scurvy, in evoking arthritic symptoms, recently described by SCHAFFENBURG and MASON (15).

## Methods

3 male albino rats, weighing from 160 to 170 gms, were injected subcutaneously with a mixture of live virulent Group A hemolytic streptococci and of homologous rat heart tissue extract. To insure a more prolonged contact time between the bacteria resp. their products and the injected host-homologous proteins — a detail considered essential in stimulating autoantibody production (15) — and wishing to obviate the danger of causing general sepsis, possibly resulting from too rapid an invasion of the circulation by the virulent streptococci, the bacterial suspension and the saline heart extract were injected mixed with agar-agar (16) to give a kind of slowly resorbable depot.

The hemolytic beta-streptococci (Group A, strain SF 135, obtained by the courtesy of Dr. P. HEDLUND, State Bacteriological Laboratory, Stockholm) were grown for 18 h. at 37°C in a calf meat's trypsin digest + nutrient broth medium, centrifuged down, washed free from the medium, resuspended in isotonic saline and standardized by dilution against a Welcome standard to give a suspension containing c:a 600 000 bacteria per ml. 2 ml of a 20 % normal rat heart extract in 0.85 % saline, prepared from a thoroughly saline-perfused rat heart by grinding with sterile sand in a mortar and clarified by 30 minutes' spinning in a Servall type SS-1 Superspeed angle centrifuge at about 13 000 × G, were mixed with 0.2 ml of the above bacterial suspension (5) and warmed in waterbath to the temperature of 42°C. 2 ml of melted 2 % agar-agar in saline, previously cooled to the same temperature, were then added, and the mixture injected subcutaneously into the back of the animals in amounts of 0.5 ml per animal. Each animal was thus inoculated with c:a 150 000 bacteria together with a quantity of soluble rat heart proteins corresponding to c:a 100 mg of fresh tissue.

A similar infection-autosensitization technique, described by HEDLUND (16) was found by this author to stimulate the production of autoantibodies against homologous renal tissue. In our experiment this technique was combined with a prolonged desoxycorticosterone acetate treatment, reported by SELYE (9) to yield a high incidence of arthritis in rats, debilitated by various, apparently relatively unspecific, operative interventions. Starting on the day of inoculation with the bacteria-heart extract mixture, each rat was daily injected with 0.2 ml of an oily suspension of desoxycorticosterone acetate, containing 15 mg crystalline DOCA per ml (i.e. 3 mg per rat and day), given subcutaneously in one dose. It was found to be more convenient to inject the hormone suspended in olive-oil than in water, as done by SELYE *et al.* (9).

For three weeks the animals were daily observed for their general behaviour and examined in respect to the eventual appearance of arthritic joint swellings. They were bled and then sacrificed on the 20th day after the inoculation with bacteria simultaneously with 3 untreated controls of the same strain, sex and closely identical body weight. The hearts and joints of the animals were examined macroscopically. Parts of the hearts, including the valves, were fixed in formalin, embedded in paraffin, sectioned, stained with hematoxylin-eosin and examined under the microscope.

The separated blood sera were tested on the presence of autoantibodies against rat heart by the collodion particle agglutination technique of CAVELTI (17) and by the agar-plate diffusion method of OUCHTERLONY (18), using a 10 % saline extract from normal rat heart (prepared in the same way as for inoculation — see above) as antigen in either case. In the case of collodion agglutination the particles were sensitized with antigen by 18 hours' incubation at + 4°C (same volumes of collodion suspension, corresponding to N:o 2 of MCFARLANDS' density scale, and of heart extract). The excess of antigen was eliminated by centrifugation and the particles were resuspended in 1.2 % saline, as recommended by CAVELTI (17). Serum titrations was performed exactly as recommended by the latter author. In the plate diffusion method (18) undiluted resp. 10 times diluted antigen was filled in both symmetrical bassins of each plate (10- resp. 1 % heart extracts) while the asymmetrical bassins were filled with undiluted test and control sera. One plate was thus needed for each serum.

## Results

None of the treated animals was found to exhibit any signs of clinical involvement of the articular system. The animals remained alert and apparently healthy for the whole duration of the experiment. The macroscopical pathological findings were likewise negative, both the hearts and joints showing a normal appearance, differing in nothing from that of the controls. At histopathological examination, diffuse proliferatively-degenerative, mostly perivascular lesions, showing some evidence of collagen necrosis (no specific collagen staining technique has been applied), were found in 2 of the 3 treated animals and in one of the 3 control animals. No significance should be ascribed to those findings, spontaneous lesions of a similar type in laboratory animals having been reported by many workers (as cited by WAKSMAN (2)).

No significant collodion particle agglutination titers were obtained — the particles were nonspecifically agglutinated by low dilutions (titers 1: 4—1: 16) of sera from both the treated and the control rats.

No specific precipitate lines were seen on the agar diffusion plates (18). Thus both serological methods, probably the most sensitive of all so far known techniques for demonstrating of the presence of antibodies, failed to detect the presence of autoantibodies against rat heart after a treatment of the animals with the latter material, combined with virulent bacteria and with DOCA overdosage.

Grateful acknowledgement is made to Ing. ROGNE ANDER for valuable technical assistance.

## Conclusions

The combined streptococcal infection + autoimmunization against homologous heart proteins + DOCA overdosage failed to duplicate the picture of human rheumatic fever in albino rats. No autoantibodies against homologous heart substance, injected in conjugation with virulent hemolytic streptococci with subsequent DOCA treatment, could be demonstrated in the blood of the animals after a period of 20 days. It seems as if the DOCA treatment had no stimulating effect upon autoantibody formation in rats (5, 6, 7) and as if the arthritic lesions found by SELYE (9) in certain DOCA-treated thyroid- or adrenalectomized rats were due to a synergistic effect of factors other than those acting in our experiment.

A further study on synergism of different rheumatogenic factors seems indicated.

REFERENCES. 1. **F. Vacirca**, Acta rheumatol. *8* (Append. 28):8 (1936). — 2. **B. H. Waksman**, Medecine *28*: 143 (1949). — 3. **F. Klinge**, Beitr. z. path. Anat. u. allg. Path. *83*: 185 (1929). — 4. **A. R. Rich** and **J. E. Gregory**, Bull. J. Hopkins Hosp. *73*: 239 a. 465 (1943); *75*: 115 (1944). — 5. **P. A. Cavelti**, Proc. Soc. Exp. Biol. a. Med. 600: 379 (1945); Arch. Path. *44*: 1 a. 13 (1947). — 6. **F. C. Bauer, Jr.**, Arch. Path. *42*: 222 (1946). — 7. **M. P. Schultz** and **E. J. Rose**, Publ. Health Repts. *62*: 1009 (1947). — 8. **W. J. Kerr**, Ann. Int. Med. *29*: 587 (1948). — 9. **H. Selye, O. Sylvester, C. E. Hall** and **C. P. Leblond**, J. A. M. A. *124*: 201 (1944). — 10. **J. F. Rinehart**, Ann. Int. Med. *9*: 586 (1935); J. Lab. a. Clin. Med. *21*: 597 (1936); — 11. **J. F. Rinehart, L. D. Greenberg, M. Olney** and **F. Choy**, Arch. Int. Med. *61*: 552 (1938). — 12. **J. F. Rinehart**, Ann. Rheum. Dis. *3*: 154 (1943); *5*: 11 (1945). — 13. **H. Selye**, Ann. Int. Med. *29*: 403 (1948). — 14. **H. v. Euler** and **L. Heller**, This Archive 2, N:o 16 (1950). — 15. **C. Schaffenburg, G. M. C. Masson** and **C. Corcoran**, Proc. Soc. Esp. Biol. a. Med. *74*: 358 (1950). — 16. **P. Hedlund**, The Scand. J. of Clin. a. Labor. Invest. *1*: 351 (1949). — 17. **P. A. Cavelti**, J. Immunol. *57*: 141 (1947). — 18. **Ö. Ouchterlony**, Arkiv f. Kemi, Mineral. och Geol. 26 B, N:o 14 (1948).

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# Demonstration of two new electrophoretic components in a serum from a patient with rheumatoid arthritis

By HELGE LAURELL

With 1 figure in the text

In pathological conditions serum components may show up which under normal circumstances are present in such small concentrations as to make their detection difficult or unlikely. In rheumatoid arthritis as well as in several other pathological conditions it is known that the hexosamine content of hydrolysed serum is elevated. During administration of ACTH (1) or cortisone (2) there is a fall in the hexosamine content of hydrolysed serum, at the same time as the clinical condition improves. It would be of interest to study the variation of the hexosamine content in various serum components which are known to contain hexosamine.

The mucoprotein M1 (3) in serum which contains about 12 % hexosamine is reported to have an isoelectric point at pH 1.8. In order to study the influence of cortisone on M1 electrophoresis was thus performed at pH 4.53. Sodium acetate buffer of ionic strength $\mu = 0.1$ was used in all experiments. After the serum had been diluted with the buffer to a final protein concentration of about 1.5 % the protein solution was dialysed against the same buffer at + 4° C for 48 hours. A slight precipitate was formed during the dialysis, and this was spun down before the electrophoresis was performed in the Tiselius apparatus using a long cell. MEHL et al (4) have shown that in certain pathological conditions it is possible to show two electrophoretic serum components which have a lower isoelectric point than albumin, if the electrophoresis is performed at pH 4.5.

The three serums were obtained from a patient with severe rheumatoid arthritis who was treated at Södersjukhuset, Stockholm. Fig. 1 shows the electrophoretic patterns before, during and after administration of cortisone. After prolonged electrophoresis the M1 component separated into two distinguishable components. Besides two other components more close to the albumin were visible before the treatment with cortisone started, one of which may correspond to M2 described in (4). It may be of interest that one of these disappeared when the patient was treated with cortisone but reappeared when cortisone was withdrawn. The great differences between the ascending and descending limbs are probably due to the same phenomenon as has been described for ovomucin (5).

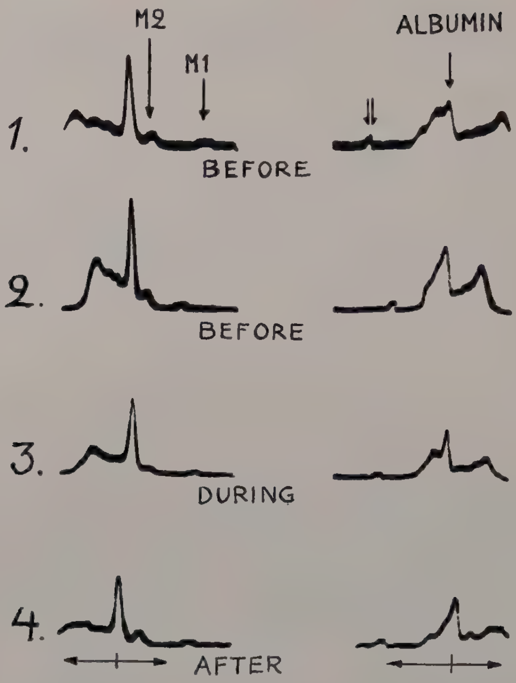

Figure 1. Electrophoretic patterns of three sera from a patient with rheumatoid arthritis before, during, and after treatment with cortisone. Anode to the right, cathode to the left. Duration of experiments 242, 162, 200 and 201 minutes respectively. The third picture was taken with $\theta = 65°$, the others with $\theta = 75°$. $\theta$ = angle of inclination of the diagonal slit; this equals zero in the vertical position.

## SUMMARY

A serum from a patient with rheumatoid arthritis is described which shows 4 electrophoretic components with an isoelectric point lower than albumin — one of the components disappears under the influence of cortisone only to reappear when the treatment had ended.

The author wishes to thank Prof. A. Tiselius for the privilege of carrying out this work in his laboratory, dr E. Jonsson, Stockholm, for placing the sera at his disposal and Fil. Kand. H. Haglund for valuable technical assistance.

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REFERENCES: (1) **Jonsson, E., Berglund, K., Hakanson, E. Y., Hävermark, N. G.,** and **Laurell, H.,** Acta Endocrinologica *4* (1950) 229. — (2) **Laurell, H.,** Nordisk Medicin (1951) in press. — (3) **Weimer, H. E., Mehl, J. W.,** and **Winzler, R. J.,** J. Biol. Chem. *185* (1950) 561. — (4) **Mehl, J. W.,** and **Golden, F.,** J. Clin. Invest. *29* (1950) 1214. — (5) **Longsworth, L. G., Cannan, R. K., MacInnes, D. A.,** J. Am. Chem. Soc. *62* (1940) 2589.

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# Optically active higher aliphatic compounds. VIII

## The synthesis of D(+)-4-methyltetracosanoic acid and D(+)-5-methylpentacosanoic acid

By STINA STÄLLBERG-STENHAGEN

In the case of the lower methyl-substituted monocarboxylic acids resolved by LEVENE and MARKER (1), sterically related acids all have the same sign of rotation (for sodium light), with the exception of the 3-methyl-substituted acids. It has been shown (2) that acids sterically related to D-glyceraldehyde are laevorotatory, except the 3-methyl-substituted acids which are dextrorotatory. The monomethyl-substituted higher acids so far described, which include acids with the methyl side chain in position 2-(3,4), 3-(4,5,6), 6-(6), 10-(7,8), and in the positions (ω–2), (ω–3) and (ω–4) (9) (ω indicating the carbon atom at the end of the chain opposite the carboxyl group), also conform to this rule. However, as the 4- and 5-methylsubstituted acids resolved by LEVENE and MARKER have a low rotation, it did not appear certain that 4- and 5-methyl-substituted higher acids with the same configuration would show a rotation of the same sign as the lower acids. Two long chain acids of this type have therefore been synthesized, *viz.* the optically active 4-methyltetracosanoic and 5-methylpentacosanoic acids sterically related to D-glyceraldehyde. The optical rotations of these acids are low, but they are both dextrorotatory for sodium light. The sign of the rotation is thus different from that of the lower acids.

The two acids (VIII and IX respectively in the chart below) were prepared by lengthening the chain of D(+)-*3-methyltricosanoic acid* (V) in the manner outlined below. The acid (V), which melts at 62.1–62.3° and has an optical rotation $[\alpha]_D^{24} + 4.22°$ (chloroform; *c*, 10), $[M]_D^{24} + 15.6°$, was prepared by lengthening the chain of L(+)-methyl hydrogen β-methylglutarate, using the method described in Part II (5). The intermediate D(—)-*3-methyl-5-ketotricosanoic acid* (IV) melts at 70.1–70.3° and has an optical rotation $(\alpha)_D^{23} - 1.35°$ (chloroform; *c*, 10), $[M]_D^{23} - 5.17°$.

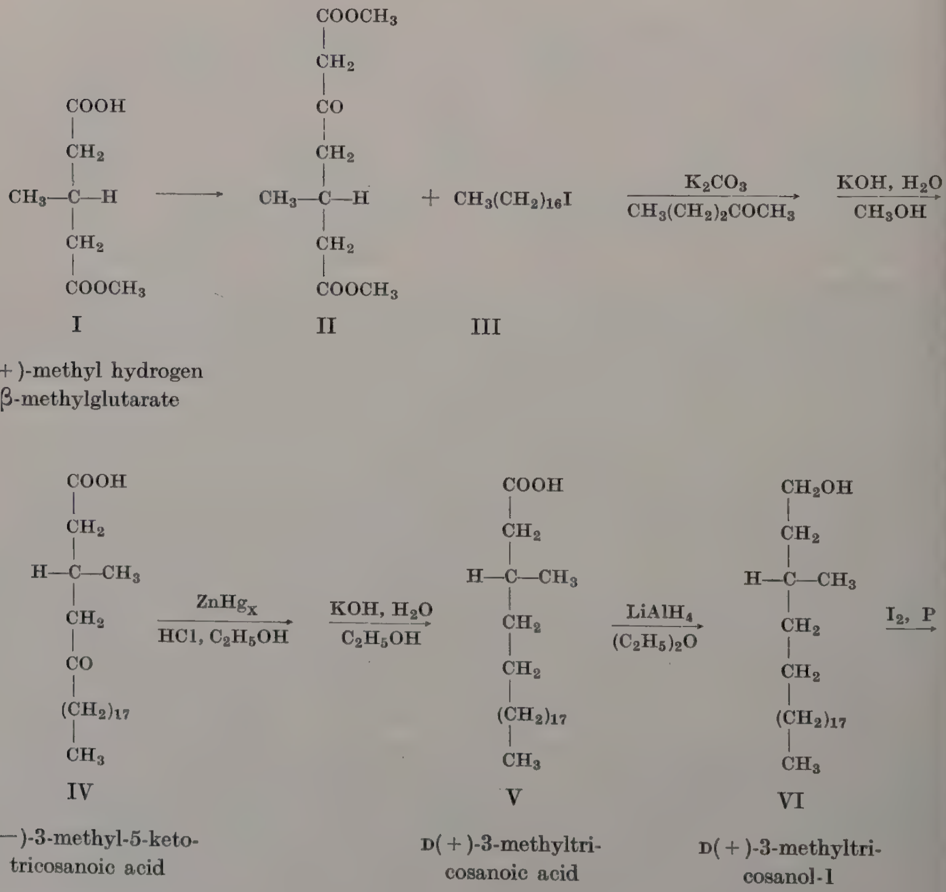
Reduction of D(+)-3-methyltricosanoic acid (V) by lithium aluminium hydride in ether solution (10) gave D(+)-*3-methyltricosanol-1* (VI) $[\alpha]_D^{23} + 2.58°$ (chloroform; *c*, 8.8), $[M]_D^{23} + 9.2°$, in a yield of 96 % of the theoretical. The alcohol has two crystalline modifications, which melt at 57.1–57.3° and 50.4–50.5° respectively. The alcohol was converted into (—)-*1-iodo-3-methyltricosane* (VII) by treatment with iodine and red phosphorus in the ordinary manner, the yield being 88 % of the theoretical. The iodide has two crystalline forms melting at 38.8–38.9° and 30.3–30.4° respectively, and has an optical rotation $[\alpha]_D^{23} - 5.96°$ (chloroform; *c*, 10), $[M]_D^{23} - 27.7°$.

The iodide (VII) gave via nitrile synthesis D(+)-*4-methyltetracosanoic acid* (VIII), $[\alpha]_{\mathrm{D}}^{23} + 0.28^\circ$ (chloroform; *c*, 9.9), $[M]_{\mathrm{D}}^{23} + 1.1^\circ$, in a yield of 91 % of the theoretical. The acid melts at 63.7–63.9°, and the corresponding *amide* at 95.6–95.9°.

D(+)-*5-Methylpentacosanoic acid* (IX), $[\alpha]_{\mathrm{D}}^{25} + 0.1^\circ$ (chloroform; *c*, 15), $[M]_{\mathrm{D}}^{25} + 0.4^\circ$, was obtained in a yield of 79 % from the iodide (VII) via malonic ester synthesis. The acid melts at 52.8–53.0°, and D-*5-methylpentacosanamide* melts at 93.3–93.5°.

Optical rotation data for the compounds VI–IX for several wave lengths in the near ultraviolet and the visible region are given in Table I. The rotations were measured with an ultraviolet polarimeter of the BRUHAT–GUINIER type.

Chart.[1]


COOH
CH2
CH3—C—H
CH2
COOCH3
I
L(+)-methyl hydrogen β-methylglutarate
COOCH3
CH2
CO
CH2
CH3—C—H
CH2
COOCH3
II
+ CH3(CH2)16I
III
K2CO3
CH3(CH2)2COCH3
KOH, H2O
CH3OH
COOH
CH2
H—C—CH3
CH2
CO
(CH2)17
CH3
IV
D(−)-3-methyl-5-keto-tricosanoic acid
ZnHgx
HCl, C2H5OH
KOH, H2O
C2H5OH
COOH
CH2
H—C—CH3
CH2
CH2
(CH2)17
CH3
V
D(+)-3-methyltri-cosanoic acid
LiAlH4
(C2H5)2O
CH2OH
CH2
H—C—CH3
CH2
CH2
(CH2)17
CH3
VI
D(+)-3-methyltri-cosanol-1
I2, P

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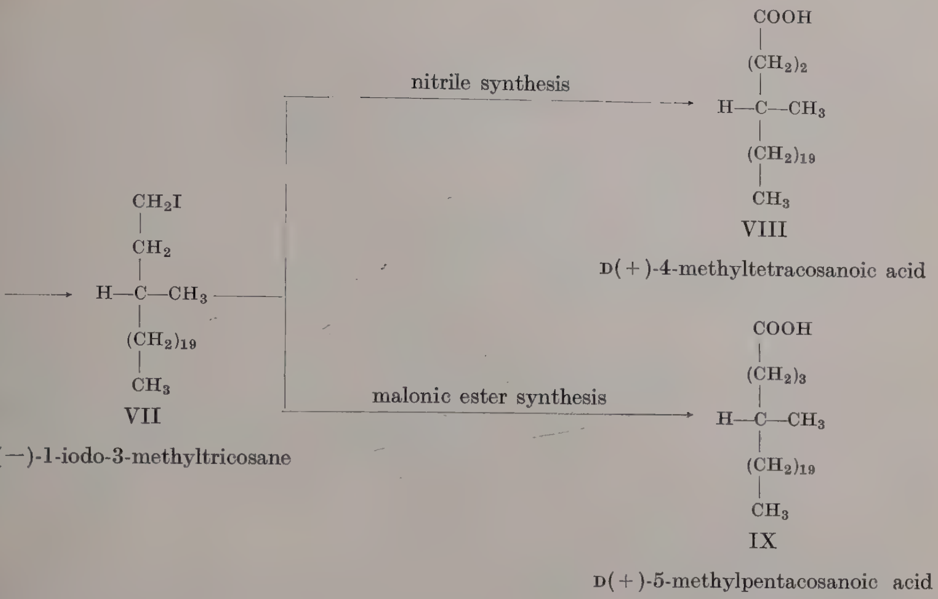
[1] The formulae are written according to FISCHER's convention.



VII

(—)-1-iodo-3-methyltricosane

VIII

D(+)-4-methyltetracosanoic acid

IX

D(+)-5-methylpentacosanoic acid

*Table I*

Molecular rotations for solutions in chloroform at 25°

| Wavelength Å | D(+)-3-Methyl-tricosanol-1<br>VI | (−)-1-Iodo-3-methyltricosane<br>VII | D(+)-4-Methyl-tetracosanoic acid<br>VIII | D(+)-5-Methylpen-tacosanoic acid<br>IX |
|---|---|---|---|---|
| | *c*, 8.82 | *c*, 10.02 | *c*, 9.92 | *c*, 13.87 |
| 5893 | + 9.3 | −27.2 | +1.1 | +0.4 |
| 5461 | +11.0 | −32.7 | +1.3 | +0.5 |
| 4358 | +18.5 | −60.7 | +1.9 | +1.0 |
| 4046 | +22.3 | −76.2 | +2.1 | +1.2 |
| 3650 | +28.8 | −110° | +2.1 | +1.4 |
| 3131 | +44.0 | | −5 | |

## Experimental[1]

D(—)-*3-Methyl-5-ketotricosanoic acid* (IV). The keto-acid was prepared by alkylation of active methyl 3-keto-5-methylpimelate (II) (derived from L(+)-methyl hydrogen β-methylglutarate (I)), with 1-iodoheptadecane (III), followed by hydrolysis and ketonic cleavage of the alkylation product. The detailed procedure has been described in Part II (5). The iodide (III) was prepared via the bromide obtained by degradation of silver stearate by bromine in carbon tetrachloride solution (12). A carefully purified homologue-free specimen of stearic acid was used as initial material. The bromide was converted into the iodide by treatment with sodium iodide in acetone solution.

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[1] Melting points, long X-ray spacings, and optical rotations were determined as described in Part I (11).

After crystallization from light petroleum (b.p. 40–60°), and from acetone, D(—)-*3-methyl-5-ketotricosanoic acid* (IV) was obtained in the form of large lustrous plates which melted at 70.1–70.3°. Previously melted material had the same m.p. The yield of pure keto-acid was 58 % of the theoretical. Long X-ray spacing (pressed specimen) 46.4 Å.

*Optical rotation* $\alpha_D^{23} - 0.54°$ (chloroform; *l*, 4; *c*, 10)
$[\alpha]_D^{23} - 1.35°$ $[M]_D^{23} - 5.17°$

*Anal.*: Calcd for $C_{24}H_{46}O_3$ (382.6) C, 75.34; H, 12.12 %
Found C, 75.73; H, 12.10 %

D(+)-*3-Methyltricosanoic acid* (V) was obtained in 69 % yield by Clemmensen reduction of the keto-acid just described. The reduction was carried out in the manner described in Part II (5). Crystallization of the reduced material from glacial acetic acid gave the acid (V) in the form of large lustrous plates of m.p. 62.1–62.3°. Long X-ray spacing (pressed specimen) 32.3 Å.

*Optical relation* $\alpha_D^{24} + 1.67°$ (chloroform; *l*, 4; *c*, 9.95)
$[\alpha]_D^{24} + 4.20°$ $[M]_D^{24} + 15.5°$

*Anal.*: Calcd for $C_{24}H_{48}O_2$ (368.6) C, 78.19; H, 13.13 %
Found C, 78.10; H, 13.06 %

D-*3-Methyltricosanamide* was prepared via the acid chloride. After crystallization from acetone and from ethanol the amide formed silky needles of m.p. 101.6–101.8°. Previously melted material usually had the same m.p., but on one occasion an m.p. of 96.6–96.9° was observed. Long X-ray spacing (pressed specimen) 33.1 Å.

*Anal.*: Calcd for $C_{24}H_{49}ON$ (367.6) N, 3.81 %
Found N, 3.80 %

D(+)-*3-Methyltricosanol-1* (VI). D(+)-3-Methyltricosanoic acid (16.0 g) was dissolved in dry ether (100 ml), and the solution added slowly to a solution of lithium aluminum hydride (*ca* 5 g) in ether (200 ml). When all had been added the mixture was refluxed for one hour. Water was added dropwise until no further reaction occurred. After an excess of dilute sulphuric acid had been cautiously added, the mixture was transferred to a separating funnel and shaken in order to dissolve the precipitate completely. The ether layer was washed twice with water, dried with sodium sulphate, and the ether distilled off. The residue melted at 57.0–57.2°, and it consisted of practically pure D(+)-*3-methyltricosanol-1*. Crystallization from ethanol gave the optically active alcohol in the form of very small lustrous crystals of m.p. 57.1–57.3°. After previous melting the m.p. was 50.4–50.5°. Long X-ray spacing (pressed specimen) 33.5 Å.

*Optical rotation* $\alpha_D^{23} + 0.91°$ (chloroform; *l*, 4; *c*, 8.82)
$[\alpha]_D^{23} + 2.58°$ $[M]_D^{23} + 9.2°$

*Anal.*: Calcd for $C_{24}H_{50}O$ (354.6) C, 81.28; H, 14.21 %
Found C, 81.23; H, 14.20 %

(—)-*1-Iodo-3-methyltricosane* (VII) The alcohol (13.1 g) was heated together with iodine (5.5 g) and red phosphorus (0.4 g) to a temperature of 145° for 4 hours. After cooling to room temperature ether was added to the reaction mixture. The ether solution was washed with water and with 5 % sodium hydroxide solution. After washing again with water, the ether solution was dried with sodium sulphate, the solvent distilled off, and the residue crystallized from a mixture of one part of benzene and five parts of ethanol. 15.2 Grams (88 % of the theoretical) of (—)-1-iodo-3-methyltricosane were obtained in the form of small lustrous plates melting at 38.5–38.7°. After two further crystallizations from the same solvent mixture, the m.p. of the iodide was 38.8–38.9°. The iodide solidified at 30.2° and remelted at 30.3–30.4°. Long X-ray spacing (pressed specimen) 30.8 Å. A melted specimen showed two sets of lines corresponding to spacings of 30.8 and 35.0 Å respectively.

*Optical rotation* $\alpha_D^{23} - 2.39°$ (chloroform; $l$, 4; $c$, 10)
$[\alpha]_D^{23} - 5.96°$ $[M]_D^{23} - 27.7°$

*Anal.*: Calcd for $C_{24}H_{49}I$ (464.6) I, 27.32 %
Found I, 26.93 %

D(+)-*4-Methyltetracosanoic acid* (VIII). The iodide (6 g), potassium cyanide (2.5 g), and 95 % ethanol (100 ml) were refluxed for 6 hours. 2.5 G of potassium cyanide were added, and the refluxing continued overnight. The mixture was acidified with dilute hydrochloric acid. Water and ether were added, and the ether layer separated, washed with water, sodium thiosulphate solution, and water. After the solution had been dried with sodium sulphate the ether was distilled off, and the residue subjected to hydrolysis by refluxing for 30 hours with a solution of potassium hydroxide (30 g) in water (30 ml) and ethanol (300 ml). The reaction mixture was worked up in the usual manner. Crystallization from light petroleum (b.p. 40–60°) gave 4.52 g (91 % of the theoretical) of D(+)-*4-methyltetracosanoic acid* of m.p. 63.6–63.8°. After recrystallization from glacial acetic acid the m.p. was 63.7–63.9°. The melting point was unchanged after previous melting. A lower melting, beautifully crystalline modification can sometimes be obtained from glacial acetic acid. This form melts at 58.5--58.8°. Long X-ray spacing (higher melting form) 47.7 Å.

*Optical rotation* $\alpha_D^{23} + 0.11°$ (chloroform; $l$, 4; $c$, 9.9)
$[\alpha]_D^{23} + 0.28°$ $[M]_D^{23} + 1.1°$

*Anal.*: Calcd for $C_{25}H_{50}O_2$ (382.7) C, 78.47; H, 13.17 %
Found C, 78.21; H, 13.17 %

D-*4-Methyltetracosanamide* was prepared by treating the acid chloride (obtained by the action of oxalyl chloride in benzene solution) with an excess of aqueous ammonia. After crystallization from methanol the amide was obtained in the form of small lustrous plates, m.p. 95.6–95.9°. The melting point was unchanged after previous melting. Long X-ray spacing (pressed specimen) 49.4 Å.

*Anal.*: Calcd for $C_{25}H_{51}ON$ (381.7) N, 3.67 %
Found N, 3.60 %

D(+)-*5-Methylpentacosanoic acid* (IX). (—)-1-Iodo-3-metyltricosane (6.4 g) was added to a solution of ethyl sodiomalonate, prepared from 6 g of ethyl malonate, 0.6 g of sodium, and 50 ml of dry ethanol, and the mixture refluxed for 6 hours. The alkylation product was isolated, and without further purification subjected to hydrolysis by refluxing for 5 hours with potassium hydroxide (15 g) in water (15 ml) and ethanol (200 ml). After acidification the substituted malonic acid was isolated and decarboxylated by heating to a temperature of 200° until all evolution of gas had ceased. After crystallization from glacial acetic acid a yield of 4.35 g (79 % of the theoretical) of monobasic acid melting at 52° was obtained. Recrystallization from light petroleum (b.p. 40–60°) gave D(+)-*5-methylpentacosanoic acid* in the form of lustrous plates of m.p. 52.8–53.0°. No polymorphism has been observed. Long X-ray spacing 47.9 Å.

*Optical rotation* $\alpha_D^{25} + 0.03°$ (chloroform; *l*, 2; *c*, 14.6)
$[\alpha]_D^{25} + 0.1°$ $[M]_D^{25} + 0.4°$

*Anal.*: Calcd for $C_{26}H_{52}O_2$ (396.7) C, 78.72; H, 13.21 %
Found C, 78.94; H, 13.18 %

D-*5-Methylpentacosanamide* melted, after crystallization from methanol, at 93.3–93.5°. The melting point was unchanged after previous melting. Long X-ray spacing (pressed specimen) 50.3 Å.

*Anal.*: Calcd for $C_{26}H_{53}ON$ (395.7) N, 3.54 %
Found N, 3.48 %

The author is indebted to Mr. L. AHLQUIST for assistance in the synthetic work. Part of the expenses involved have been met by a grant from the Swedish National Anti-tuberculosis Association.

## SUMMARY

D(+)-4-Methyltetracosanoic acid and D(+)-5-methylpentacosanoic acid have been synthesized by lengthening the chain of D(+)-3-methyltricosanoic acid. It is found that these acids have a low optical rotation, and that the sterically related 3-, 4-, and 5-methyl-substituted acids have a rotation of the same sign for sodium light.

Institute of Medical Chemistry, University of Uppsala.

REFERENCES: 1. **Levene, P. A.**, and **Marker, R. E.**, J. Biol. Chem. *98*, 1 (1932). — 2. **Ställberg-Stenhagen, S.**, and **Stenhagen, E.**, Arkiv för Kemi, Min. o. Geol. *24 B* No. 8 (1947). — 3. **Ställberg-Stenhagen, S.**, *ibid.* *23 A*, No. 15 (1946). — 4. *Idem*, Arkiv för Kemi *1*, Nr. 18 (1949). — 5. *Idem*, Arkiv för Kemi, Min. o. Geol. *26 A*, No. 1 (1948). — 6. *Idem*, Arkiv för Kemi, in the press. — 7. *Idem*, Arkiv för Kemi, Min. o. Geol. *26 A*, No. 12 (1948). — 8. **Prout, S. F.**, **Cason, J.**, and **Ingersoll, A. W.**, J. Amer. Chem. Soc. *70*, 298 (1948). — 9. **Ställberg-Stenhagen, S.**, Arkiv för Kemi *2*, 95 (1950). — 10. **Nystrom, R. F.**, and **Brown, W. G.**, J. Amer. Chem. Soc. *69*, 2548 (1947). — 11. **Ställberg-Stenhagen, S.**, Arkiv för Kemi, Min. o. Geol. *23 A*, No. 15 (1946). — 12. **Hunsdiecker, H.**, and **Hunsdiecker, Cl.**, Berichte *75*, 291 (1942).

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# Studies on synthetic growth substances. I

By Arne Fredga

Among the synthetic plant hormones, compounds like naphthalene-acetic acid and naphthoxy-acetic acid have been extensively studied. Naphthalene derivatives with sulphur or selenium in the side chain, however, have not, to my knowledge, been investigated from this point of view. Such compounds are readily accessible and the structure of the side chain can easily be varied in different ways. If these acids are biologically active, they may be utilised for studying the effects of branching and variations in length of the side chain.

Some years ago, Kögl reported (1), that the optical antipodes of the α-(3-indolyl)-propionic acid had very different biological effect. The naphthalene compounds seem to form suitable material for a more extensive study of such sterical effects. Especially it would be of interest to see if sterically related compounds have analogous biological effects. If that is so, a study of the biological effects may in certain cases permit conclusions to be drawn as to the sterical relationships of optically active compounds.

After the beginning of the present investigation, Erdtman and Nilsson (2) reported that certain phenylsulphide-acetic acids possess herbicidal properties, similar to those of the corresponding phenoxy compounds.

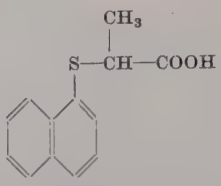
Naphthylsulphide-carboxylic acids are easily obtained from naphthyl mercaptans and halogenesubstituted acids. A few derivatives of acetic and propionic acid have previously been prepared in this way (3). The reaction between naphthyldiazonium compounds and mercapto acids can also be utilised (4) but with less advantage.

Acids with a sulphur atom in the side chain may also be prepared from naphthylmethyl halides and mercapto acids. This method also permits the preparation of acids with selenium in the side chain.

In this paper, eight sulphur-containing acids of different types (I–VIII) are described. Their biological properties are being investigated by Dr. Börje Åberg (Royal Agricultural College, Uppsala). The results of some experiments with α-(1-naphthylmethyl-sulphide)-propionic acid have already been published (5).

## Experimental

**α-(1-Naphthylsulphide)-propionic acid (I).** 6.4 g (0.04 mol) 1-naphthyl mercaptan and 3.4 g (0.085 mol) sodium hydroxide were dissolved in 10 ml water and 6.45 g (0.042 mol) of α-bromopropionic acid, dissolved in 40 ml water, were added. The mixture was shaken until homogeneous and left to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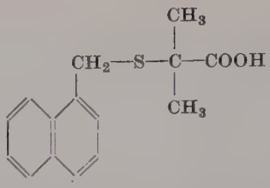
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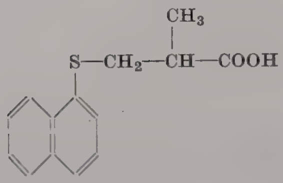
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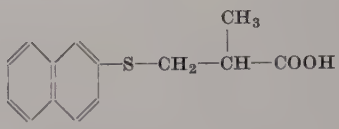
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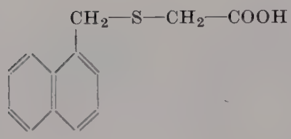
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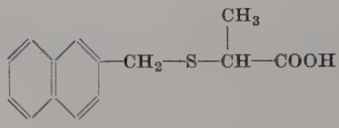
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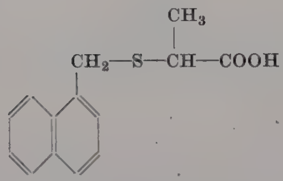
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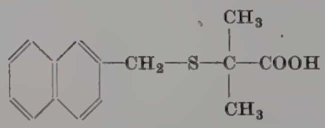
(VIII)

stand for 36 hours. On acidifying with hydrochloric acid the product separated as an oil, which gradually crystallised. After drying it was recrystallised four times, alternately from cyclohexane and formic acid. The yield of crude product was 9.0 g (96 %) and after the recrystallisations 5.0 g remained. The acid forms short prisms with m. p. 69–70.5°.

0.2928 g sbst: 11.86 ml 0.1065 *N* NaOH. — 21.41 mg sbst: 52.62 mg $CO_2$ and 9.97 mg $H_2O$. — 38.33 mg sbst: 4.910 ml 0.06725 *N* NaOH.

| | | | | | |
|---|---|---|---|---|---|
| $C_{13}H_{12}O_2S$ | Calc. | Equiv. wt. 232.3 | C 67.21 | H 5.21 | S 13.80 |
| | Found | » » 231.8 | » 67.07 | » 5.21 | » 13.81 |

**β-(1-Naphthylsulphide)-*iso*-butyric acid (II).** The acid was prepared from 6.4 g (0.04 mol) 1-naphthyl mercaptan and 6.7 g (0.04 mol) β-bromo-*iso*-butyric acid in the same way as described above. The yield of crude product was nearly quantitative. As the acid is readily soluble in most solvents and crystallises slowly, the purification was rather tedious and much material was lost. After dissolving in bicarbonate solution and reprecipitation with hydrochloric acid and three recrystallisations from cyclohexane, 3.1 g of pure acid were obtained as aggregates of small prisms with m. p. 58–60°.

0.2899 g sbst: 11.05 ml 0.1065 *N* NaOH. — 27.98 mg sbst: 70.07 mg $CO_2$ and 13.77 mg $H_2O$. — 40.11 mg sbst: 4.855 ml 0.06725 *N* NaOH.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_{14}H_{14}O_2S$ | Calc. | Equiv. wt. | 246.3 | C 68.26 | H 5.73 | S 13.02 |
| | Found | » | » 246.3 | » 68.34 | » 5.51 | » 13.05 |

**1-Naphthylmethyl-sulphide-acetic acid (III).** 9.5 g (0.1 mol) thioglycolic (mercaptoacetic) acid and 10 g (0.25 mol) sodium hydroxide were dissolved in 75 ml water and a solution of 17.7 g 1-naphthylmethyl chloride (6) in 75 ml alcohol was added. An emulsion was formed, but after shaking for a few minutes a homogeneous solution was obtained. Soon the sodium salt of the acid began to crystallise as glistening plates, which gradually filled the whole solution. After 24 hours, the salt was filtered off and recrystallised from dilute (50 %) methanol. It holds two molecules of water. Yield 18.7 g.

0.5688 g sbst: 0.1393 g $Na_2SO_4$. — 0.6293 g sbst: 0.0772 g $H_2O$.

| | | | |
|---|---|---|---|
| $C_{13}H_{11}O_2NaS$, 2 $H_2O$ (290.3) | Calc. | Na 7.92 | $H_2O$ 12.41 |
| | Found | » 7.93 | » 12.27 |

11 g of the salt were dissolved in 400 ml of hot water and the acid was precipitated by hydrochloric acid; it separated as an oil, which readily crystallised. The yield was 8.9 g; after two recrystallisations from carbon tetrachloride, 7.4 g of pure acid were obtained as small glistening crystals of rhombohedral habit. M. p. 112.5–113.5°.

0.2501 g sbst: 10.09 ml 0.1065 *N* NaOH. — 39.24 mg sbst: 5.036 ml 0.06725 *N* NaOH.

| | | | | |
|---|---|---|---|---|
| $C_{13}H_{12}O_2S$ | Calc. | Equiv. wt. | 232.3 | S 13.80 |
| | Found | » | » 232.8 | » 13.83 |

**α-(1-Naphthylmethyl-sulphide)-propionic acid (IV).** 11.0 g (0.104 mol) thiolactic (α-mercaptopropionic) acid and 9.0 g (0.225 mol) sodium hydroxide were dissolved in 100 ml of water and a solution of 17.7 g (0.1 mol) 1-naphthylmethyl chloride (6) in 100 ml alcohol was added. A homogeneous solution was obtained after shaking for 20 minutes. After 24 hours the alcohol was blown off with gentle heating, the solution was made up to 200 ml with water and filtered with charcoal. The acid was then precipitated by hydrochloric acid and obtained as a readily crystallising oil. The yield of crude product was practically quantitative. The acid was recrystallised several times alternately from formic acid and carbon tetrachloride and obtained as small prisms with m. p. 112–113°.

0.2800 g sbst: 10.66 ml 0.1065 *N* NaOH. — 23.12 mg sbst: 57.83 mg $CO_2$ and 11.47 mg $H_2O$. — 27.33 mg sbst: 25.94 mg $BaSO_4$.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_{14}H_{14}O_2S$ | Calc. | Equiv. wt. | 246.3 | C 68.26 | H 5.73 | S 13.02 |
| | Found | » | » 246.6 | » 68.26 | » 5.55 | » 13.03 |

**α-(1-Naphthylmethyl-sulphide)-*iso*-butyric acid (V).** 8.4 g (0.04 mol) of α-ethylxanthogene-*iso*-butyric acid (7) were dissolved in 150 ml of concentrated aqueous ammonia. After standing for two days, excess of ammonia and xanthogene amide were removed (7) and 3.2 g (0.08 mol) of sodium hydroxide were added. The resulting solution, containing the secondary sodium salt of α-mercapto-*iso*-butyric acid, was mixed with a solution of 7.1 g (0.04 mol) 1-naphthylmethyl chloride in 75 ml alcohol. After shaking for several hours and standing for two days, the reaction product was isolated as described for α-(1-naphthylmethyl-sulphide)-propionic acid (IV). The yield of crude product was 9.5 g (91 %); after one recrystallisation from formic acid and three from cyclohexane, 7.6 g of pure acid were obtained as small prisms with m. p. 116–117°.

0.3106 g sbst: 11.91 ml 0.1065 *N* NaOH. — 31.37 mg sbst: 79.46 mg $CO_2$ and 16.56 mg $H_2O$. — 37.20 mg sbst: 4.230 ml 0.06725 *N* NaOH.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_{15}H_{16}O_2S$ | Calc. | Equiv. wt. | 260.3 | C 69.20 | H 6.19 | S 12.32 |
| | Found | » » | 260.6 | » 69.12 | » 5.91 | » 12.26 |

**β-(2-Naphthylsulphide)-*iso*-butyric acid (VI).** 4.8 g (0.03 mol) 2-naphthyl mercaptan and 2.65 g (0.066 mol) sodium hydroxide were dissolved in 50 ml water and 5.5 g (0.033 mol) of β-bromo-*iso*-butyric acid were added. After shaking and standing for two days, the reaction product was isolated as described above and the acid was recrystallised several times from dilute acetic acid. It forms glistening plates or shales with m. p. 88.5–89.5°.

0.2969 g sbst: 11.31 ml 0.1065 *N* NaOH. — 28.59 mg sbst: 71.67 mg $CO_2$ and 14.03 mg $H_2O$. — 36.88 mg sbst: 4.478 ml 0.06725 *N* NaOH.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_{14}H_{14}O_2S$ | Calc. | Equiv. wt. | 246.3 | C 68.26 | H 5.73 | S 13.02 |
| | Found | » » | 246.5 | » 68.41 | » 5.49 | » 13.09 |

**α-(2-Naphthylmethyl-sulphide)-propionic acid (VII).** 4.3 g (0.04 mol) thiolactic (α-mercaptopropionic) acid and 3.5 g (0.087 mol) sodium hydroxide were dissolved in 50 ml water and 8.9 g 2-naphthylmethyl bromide (8), dissolved by gentle heating in 100 ml alcohol, were added. After shaking for several hours and standing for two days, the reaction product was isolated in the usual way. The yield of crude product was 8.6 g (87 %); after two recrystallisations from formic acid 4.8 g were obtained as small rectangular plates with m. p. 95.5–96.5°.

0.2884 g sbst: 11.00 ml 0.1065 *N* NaOH. — 28.44 mg sbst: 71.21 mg $CO_2$ and 14.29 mg $H_2O$. — 51.70 mg sbst: 6.275 ml 0.06725 *N* NaOH.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_{14}H_{14}O_2S$ | Calc. | Equiv. wt. | 246.3 | C 68.26 | H 5.73 | S 13.02 |
| | Found | » » | 246.2 | » 68.33 | » 5.62 | » 13.08 |

**α-(2-Naphthylmethyl-sulphide)-*iso*-butyric acid (VIII).** The acid was prepared in the same way as V, starting from 10.5 g (0.05 mol) α-ethylxanthogene-*iso*-butyric acid, 4.0 g (0.1 mol) sodium hydroxide and 11.0 g (0.05 mol) 2-naphthylmethyl bromide. The yield of crude product was 11.4 g (88 %). After recrystallisation from formic acid, carbon tetrachloride and once more from formic acid, 7.7 g of pure acid were obtained as glistening needles with m. p. 144.5–145.5°.

0.3077 g sbst: 11.11 ml 0.1065 *N* NaOH. — 23.25 mg sbst: 58.86 mg $CO_2$ and 12.35 mg $H_2O$. — 47.14 mg sbst: 5.365 ml 0.06725 *N* NaOH.

| | | | | | |
|---|---|---|---|---|---|
| $C_{15}H_{16}O_2S$ | Calc. | Equiv. wt. 260.3 | C 69.20 | H 6.19 | S 12.32 |
| | Found | » » 260.1 | » 69.09 | » 5.94 | » 12.27 |

The author is indebted to Mr A. Brändström for most of the analyses and to several junior students in the laboratory for preparing naphthyl mercaptanes and naphthylmethyl halides.

## SUMMARY

A number of naphthalene derivatives (I–VIII) containing a sulphur atom and a carboxyl group in the side chain have been prepared. Compounds of this type may form suitable material for studying the influence of structural and sterical factors on the biological activity of naphthalene derivatives.

REFERENCES. 1) **F. Kögl** and **B. Verkaaik,** Z. physiol. Chem. **280**, 167 (1944). — 2) **H. Erdtman** and **G. Nilsson,** Acta Chem. Scand. **3**, 901 (1949). — 3) **P. Friedländer** and **N. Woroshzow,** Ann. **388**, 14 (1913). — **F. Krollpfeiffer** and **H. Schultze,** Ber. **56**, 1819 (1923). — 4) **Kalle & Co,** Chem. Centr. **1908:1**, 1221 and **1925:2**, 773. — 5) **B. Åberg,** Physiologia plantarum **3**, 447 (1950). — 6) Organic Reactions **1**, 70 (1939). — 7) **E. Biilmann,** Ann. **348**, 128 (1906). — 8) **S. C. J. Olivier** and **J. Wit,** Rec. trav. chim. **57**, 90 (1938).

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# On the conditions for the formation of quasi-racemic compounds.

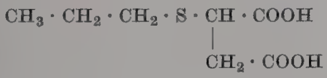
## I. S-Propylmercaptosuccinic acid and S-methylmercaptoethylsuccinic acid

By Magnus Matell.

With 3 figures in the text

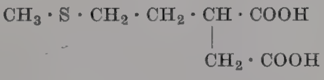
The method of quasi-racemates for the establishment of configurational relations between optically active compounds (1) has been successfully applied to a number of steric problems, especially by Fredga and collaborators (2). The preparation of optically active compounds often is laborious and timeconsuming and so it is of great importance to know in which systems quasi-racemic compounds are likely to occur. There seems to be many factors influencing the formation of such compounds; therefor the author during the last years has studied several substituted succinic acids with this point in view (3).

According to Lettré one condition for the formation of quasi-racemic compounds is that the structural differences between the components must not exceed the limits of isomorphous exchangeability (4). As it has been shown that $CH_2$ can be isomorphously replaced by S it was of interest to study the influence of such a structural change on the formation of quasi-racemates. On this problem there are already some results available (3, 5, 6, 7). Following a suggestion by Professor A. Fredga the present author studied S-propylmercaptosuccinic acid (I) and S-methylmercaptoethylsuccinic acid (II), where one $CH_2$ and one S have changed places.



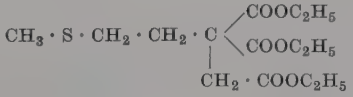
$$CH_3 \cdot CH_2 \cdot CH_2 \cdot S \cdot \underset{\displaystyle CH_2 \cdot COOH}{\underset{|}{CH}} \cdot COOH$$

I



$$CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot \underset{\displaystyle CH_2 \cdot COOH}{\underset{|}{CH}} \cdot COOH$$

II



III

Neither of these seems to have been described in literature before. Fredga, however, has prepared *rac.*-S-propylmercaptosuccinic acid and (+)-S-propylmercaptosuccinic acid from *rac.*-mercaptosuccinic acid resp. (+)-mercaptosuccinic acid and propyl halide (private communication).

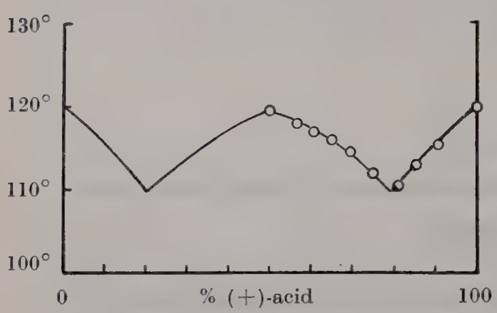


Fig. 1. (+)-and (—)-S-propylmercaptosuccinic acid.

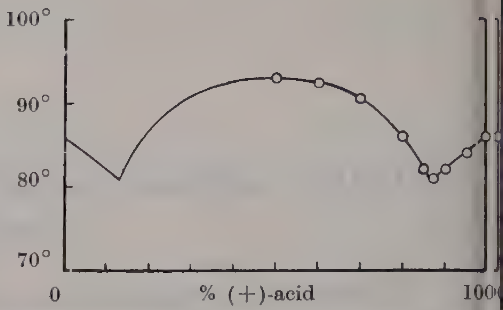


Fig. 2. (+)-and (—)-S-methylmercaptoethylsuccinic acid.

*rac.*-S-Propylmercaptosuccinic acid was prepared from bromosuccinic acid and propylmercaptan and was resolved into optical antipodes. The (+)-acid thus obtained had the same properties as the (+)-acid prepared by FREDGA ($[\alpha]^{18}_{\mathrm{D}}$ = + 144°7; M. p. 119°).

*rac.*-S-Methylmercaptoethylsuccinic acid was obtained from ethyl ethan-1.1.2-tricarboxylate, sodium ethoxide and β-chloroethylmethylsulfide, the product (III) was hydrolyzed and decarboxylated, giving II, which was resolved into optical antipodes.

The inactive acids are true racemic compounds as can be seen from fig. 1—2 and table 1. The racemate-formation tendencies are intermediate and consequently the acids with opposite configurations may form a quasi-racemic compound though the structural difference perhaps makes the existence of such a compound less probable.

*Table 1.*

Thermal data obtained by the contact method

| Components | M. p. | Eutectic temp. | Molecular compound M. p. |
|---|---|---|---|
| (+)-S-Propylmercaptosuccinic acid | 120°5 | 108° | — |
| *rac-* » » | 119° | | |
| (+)-S-Methylmercaptoethylsuccinic acid | 86° | 78° | — |
| *rac.-* » » | 94° | | |
| (+)-S-Propylmercaptosuccinic acid | 120°5 | 97° | 103° |
| (—)-S-Methylmercaptoethylsuccinic acid | 85°5 | 79° | |
| (+)-S-Propylmercaptosuccinic acid | 120°5 | 69° | — |
| (+)-S-Methylmercaptoethylsuccinic acid | 86° | | |

Preliminary examinations by means of the KOFLER contact method (8) showed, however, that I and II with opposite directions of rotation formed a molecular compound while the acids with the same direction of rotation did

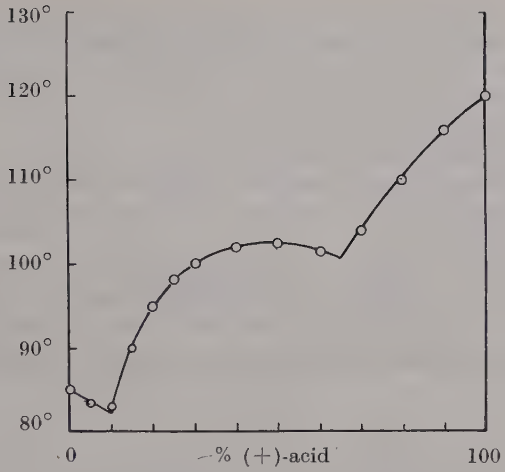


Fig. 3. (+)-S-propylmercaptosuccinic acid and (—)-S-methylmercaptoethylsuccinic acid.

not (table 1). As can be seen from fig. 3 this compound is formed in proportions 1:1 and so it was concluded to be a quasi-racemic compound.

It may further be concluded that the antipodes of the compounds I and II with opposite directions of rotation also have opposite configurations. The present author in another investigation found that (+)-S-propylmercaptosuccinic acid has the same configuration as D-glyceraldehyde (3) and consequently (+)-S-methylmercaptoethylsuccinic acid has D-configuration too.

## Experimental

### S-Propylmercaptosuccinic acid

***rac*.-S-Propylmercaptosuccinic acid.** 59 g (0.3 mol) bromosuccinic acid dissolved in 100 ml water was neutralized with sodiumcarbonate, 25 g (0.33 mol) propylmercaptan and 14 g sodiumhydroxide in 60 ml water was added and the mixture placed in a refrigerator for three days. The liquid was acidified with 100 ml dilute sulphuric acid (1:1) and extracted several times with 50 ml portions of ether. After evaporation the product was boiled with 700 ml benzene, the solution was then filtered and evaporated to a small volume from which after cooling the acid separated as very fine and colourless crystals (45 g). The crude acid was recrystallized several times from ethyl acetate + petroleum ether and finally from water. 31 g acid was obtained. M. p. 118—119°. It is soluble in water, alcohol, ethyl acetate, ether, acetone and chloroform, less soluble in benzene, insoluble in petroleum ether.

*Analysis*: 67.70 mg acid: 7.175 ml 0.0977-*N* NaOH

33.37 » » : 53.34 mg $CO_2$ and 18.35 mg $H_2O$

54.69 » » : 8.560 ml 0.0662-N NaOH (combustion according to *Grote-Krekeler*)

$C_7H_{12}O_4S$ calc E. W. 96.1; C 43.73; H 6.29; S 16.68

found » 96.6; » 43.63; » 6.15; » 16.62

**Resolution of S-propylmercaptosuccinic acid.** Preliminary experiments on resolution showed that the (+)-acid could be obtained from the neutral quinine salt and the acid cinchonidine salt, but the neutral strychnine salt crystallized from dilute alcohol gave the best result. The (—)-acid could be obtained from the neutral strychnine and brucine salts, the former crystallized from water gave the best result.

30 g (0.16 mol) *racem*-acid was dissolved in 300 ml water and 350 ml alcohol. The solution was brought to the boiling point and 100 g (0.30 mol) strychnine was added. A small quantity of strychnine did not dissolve and was removed by filtering. The solution was allowed to stand for 6 hours in a refrigerator giving 61 g salt which was recrystallized as is shown by the scheme below; the optical activity *of the acid* was measured in acetone.

| Crystallization | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| ml alcohol | 350 | 165 | 100 | 80 | 40 | 30 |
| ml water | 300 | 135 | 100 | 80 | 40 | 30 |
| g salt | 61 | 40 | 34.5 | 28.5 | 25.5 | 24 |
| $[\alpha]_D^{25}$ | +84° | +118° | +132° | +133° | — | +133° |

The mother liquor from crystallization 1 was boiled to remove the alcohol, diluted with water to 800 ml and heated to boiling. The solution was placed in a refrigerator for one day giving 40 g salt which was recrystallized several times from hot water, the optical activity *of the acid* being measured in acetone. The following series of crystallizations was obtained.

| Crystallization | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| ml water | 800 | 600 | 300 | 200 | 100 | 75 | 75 | 60 | 60 |
| g salt | 40 | 37 | 18 | 13.5 | 12 | 11 | 10.5 | 8 | 7 |
| $[\alpha]_D^{25}$ | —76°5 | —83° | —118°5 | —122° | — | —128° | — | —129°5 | |

Owing to shortage of substance the series was broken before maximum rotatory power was attained.

The salts were treated with sodium carbonate, the strychnine removed and the acids liberated with dilute sulphuric acid and extracted with ether.

**(+)-S-Propylmercaptosuccinic acid.** The crude acid (4.0 g) was recrystallized from ethyl acetate + petroleum ether. Yield 2.2 g. M. p. 118.5—119°5.

*Analysis:* 69.08 mg acid: 7.375 ml 0.0977-N NaOH
31.62 » » : 50.89 mg $CO_2$ and 18.11 mg $H_2O$
$C_7H_{12}O_4S$ calc. E. W. 96.1; C 43.73; H 6.29
found » 95.9; » 43.92; » 6.41
0.0878 g acid in *acetone*, made up to 10.00 ml:
$\alpha_D^{18} = + 1°265$; $[\alpha]_D^{18} = + 144°1$; $[M]_D^{18} = + 277°0$
0.0932 g acid in *ethyl acetate*, made up to 10.00 ml:
$\alpha_D^{18} = + 1°385$; $[\alpha]_D^{18} = + 148°6$; $[M]_D^{18} = + 285°7$

0.1011 g acid in *chloroform*, made up to 10.00 ml:
$\alpha_{D}^{20} = +1^{\circ}.365$; $[\alpha]_{D}^{20} = +135^{\circ}.0$; $[M]_{D}^{20} = +259^{\circ}.5$
0.0982 g acid in *alcohol*, made up to 10.00 ml;
$\alpha_{D}^{20} = +1^{\circ}.347$; $[\alpha]_{D}^{20} = +137^{\circ}.2$; $[M]_{D}^{20} = +263^{\circ}.7$

**Strychnine salt of (+)-S-propylmercaptosuccinic acid.** Crystallization from dilute alcohol yielded colourless plates with three moles of water of crystallization.

*Analysis*: 0.5600 g salt: loss in weight over $P_2O_5$ 0.0330 g
$C_7H_{12}O_4S$, $2C_{21}H_{22}O_2N_2$, $3H_2O$ calc. $H_2O$ 5.91
found » 5.89
0.1497 g dry salt: 8.385 ml $N_2$ at $19^{\circ}.7$ and 764.9 mm
$C_7H_{12}O_4S$, $2C_{21}H_{22}O_2N_2$ calc. N 6.51
found » 6.58

**(—)-S-Propylmercaptosuccinic acid.** The crude acid (1.9 g) was recrystallized from ethyl acetate + petroleum ether. Yield 1.4 g. M. p. 115—117°.5.

*Analysis*: 60.64 mg acid: 6.500 ml 0.0977-N NaOH
31.26 » » : 49.63 mg $CO_2$ and 17.40 mg $H_2O$
$C_7H_{12}O_4S$ calc. E. W. 96.1; C 43.73; H 6.29
found » 95.5; » 43.33; » 6.23
0.1006 g acid in *acetone*, made up to 10.00 ml:
$3\,\alpha_{D}^{25} = -3^{\circ}.968$; $[\alpha]_{D}^{25} = -131^{\circ}.5$; $[M]_{D}^{25} = -252^{\circ}.8$

The rotatory power shows that the product was not quite pure but contained about 4 % of the other antipode. For experiments on quasi-racemate formation this has little significance.

**Strychnine salt of (—)-Propylmercaptosuccinic acid.** Crystallization from water yielded a white, crystalline powder with four moles of water of crystallization.

*Analysis*: 0.4113 g salt: loss in weight over $P_2O_5$ 0.0311 g
$C_7H_{12}O_4S$, $2C_{21}H_{22}O_2N_2$, $4H_2O$ calc. $H_2O$ 7.72
found » 7.56
0.1770 g dry salt: 9.975 ml $N_2$ at $21^{\circ}.4$ and 767.6 mm
$C_7H_{12}O_4S$, $2C_{21}H_{22}O_2N_2$ calc. N 6.51
found » 6.60

### S-Methylmercaptoethylsuccinic acid

**Ethyl butane-4-methylsulfide-1.1.2-tricarboxylate** (III). 11.5 g (0.5 gram-atom) sodium was dissolved in 225 ml absolute alcohol. 123 g (0.5 mol) ethyl ethane-1.1.2-tricarboxylate (9) was added while stirring and after a few minutes 55 g (0.5 mol) β-chloroethylmethylsulfide (10) was slowly added. The reaction was initiated by gentle heating with a small flame. After the reaction ceased the mixture was refluxed for one hour. The alcohol was distilled off, the residue shaken with equal volume water and the oily layer separated, washed with water and dried with anhydrous sodium sulfate. The ester was fractionated *in vacuo* through a WIDMER column. The main fraction (96 g) passed over at 187—188°/11 mm. It had a faint yellow colour and a slight, very disagreeable smell. $n_D^{17} = 1.4637$.

***rac*.-S-Methylmercaptoethylsuccinic acid.** 90 g ethyl butane-4-methylsulfide-1.1.2-tricarboxylate was heated on a water bath with excess of alcoholic potash. In a short time the potassium salt separated and was filtered off. The salt was treated with dilute sulphuric acid and the acid extracted with ether. On evaporation 54 g was obtained. This product was decarboxylated by heating at 130° as long as carbon dioxide was evolved. When cooling the product crystallized and was recrystallized with charcoal from benzene. After recrystallization from ethyl acetate + petroleum ether 26 g pure acid was collected. M. p. 92—93°. The compound is soluble in water, alcohol, ethyl acetate, acetone, ether and chloroform, less soluble in benzene and insoluble in petroleum ether.

*Analysis*: 0.2063 g acid: 21.98 ml 0.0977-N NaOH
31.57 mg » : 50.44 mg $CO_2$ and 17.49 mg $H_2O$
51.16 » » : 7.980 ml 0.0662-N NaOH (combustion according to *Grote-Krekeler*)
$C_7H_{12}O_4S$ calc. E. W. 96.1; C 43.73; H 6.29; S 16.68
found » 96.1; » 43.59; » 6.20; » 16.57

**Resolution of S-methylmercaptoethylsuccinic acid.** Preliminary experiments on resolution showed that the (+)-acid could be obtained from the neutral strychnine, cinchonidine and quinidine salts, strychnine giving the best result. The (—)-acid could be obtained from the neutral quinidine salt.

18.4 g (0.1 mol) *racem*-acid was dissolved in 800 ml water. 64 g (0.2 mol) strychnine was added to the boiling solution, which was filtered to remove some undissolved strychnine. After one day the salt (30 g) was removed and recrystallized from hot water, the optical activity of samples *of the acid* being measured in acetone. The following series of crystallizations was obtained.

| Crystallization | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| ml water | 800 | 200 | 175 | 150 | 150 |
| g salt | 30 | 24 | 21.5 | 15 | 11.5 |
| $[\alpha]_D^{25}$ | +27° | +34.°5 | +35.°5 | +36.°5 | +36.°5 |

The mother liquor from crystallization 1 was treated with sodium carbonate, the strychnine removed and the acid liberated with dilute sulphuric acid. The acid was extracted with ether, which after evaporation left a residue of 9 g partially active acid. $[\alpha]_D^{25} = -16°$ (in acetone). This acid (0.05 mol) was dissolved in 125 ml acetone and 125 ml water, 35.5 g (0.1 mol) quinidine was added during boiling and the solution placed in a refrigerator over night. The salt (32 g) was recrystallized from dilute acetone. The following series of crystallizations was obtained; the rotatory power *of the acid* was measured in acetone

| Crystallization | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| ml acetone | 125 | 75 | 50 | 50 | 50 | 50 | 40 | 40 | 40 |
| ml water | 125 | 75 | 50 | 50 | 50 | 50 | 40 | 40 | 40 |
| g salt | 32 | 21 | 19.5 | 17 | — | 15 | — | 12 | 11 |
| $[\alpha]_D^{25}$ | —26°5 | —29° | — | —32° | — | —34° | — | —35°5 | — |

The (+)-acid was liberated from its salt after removing the strychnine. The (—)-acid was obtained directly from its salt by acidifying with dilute sulphuric acid and extraction with ether in the usual manner.

**(+)-S-Methylmercaptoethylsuccinic acid.** The crude acid was recrystallized from ethyl acetate + petroleum ether. Yield 1.3 g. M. p. 84.5—85.°5.

*Analysis*: 74.27 mg acid: 7.870 ml 0.0977-N NaOH
28.27 » » : 45.36 mg $CO_2$ and 16.45 mg $H_2O$
$C_7H_{12}O_4S$ calc. E. W. 96.1; C 43.73; H 6.29
found » 96.6; » 43.78; » 6.51
0.0996 g acid in *acetone*, made up to 10.00 ml:
$3\,\alpha_D^{25} = +1^\circ16$; $[\alpha]_D^{25} = +38^\circ8$; $[M]_D^{25} = +74^\circ6$

**Strychnine salt of (+)-S-methylmercaptoethylsuccinic acid.** Crystallization from water yielded colourless plates with varying content of water.

*Analysis*: 0.0963 g dry salt: 5.96 ml $N_2$ at 18°5 and 737.2 mm
0.1013 » » » : 6.14 » » » 18°1 » 747.5 »
0.1533 » » » : 9.45 » » » 20°2 » 745.3 »
$C_7H_{12}O_4S, 2C_{21}H_{22}O_2N_2$ calc. N 6.51
$C_7H_{12}O_4S, 3C_{21}H_{22}O_2N_2$ calc. N 7.03
found » 7.03; 6.99; 7.04

From the analysis data it is evident that the substance obtained after recrystallization 5 of the mixture of S-methylmercaptoethylsuccinic acid and strychnine was not the pure neutral salt. The compound $C_7H_{12}O_4S, 3C_{21}H_{22}O_2N_2$ on the other hand seems less probable in spite of the fact that it is in good agreement with the analysis. In the resolution of n-butylsuccinic acid and *iso*-butylsuccinic acid FREDGA and SAHLBERG noted that the strychnine salts were partially hydrolyzed on recrystallization (11). It is possible that the same phenomenon is responsible to the abovementioned complication in which case the consistency between the nitrogen content found and that calculated for the compound $C_7H_{12}O_4S, 3C_{21}H_{22}O_2N_2$ is a mere chance.

**(—)-S-Methylmercaptoethylsuccinic acid.** The acid was recrystallized from ethyl acetate + petroleum ether. Yield 1.6 g. M. p. 84.5—85°5.

*Analysis*: 64.95 mg acid: 6.855 ml 0.0977-N NaOH
30.00 » » : 48.22 mg $CO_2$ and 17.26 mg $H_2O$
$C_7H_{12}O_4S$ calc. E. W. 96.1; C 43.73; H 6.29
found » 97.0; » 43.86; » 6.44

0.0939 g acid in *acetone*, made up to 10.00 ml:
$3\ \alpha_D^{25} = -1\overset{\circ}{.}088;\ [\alpha]_D^{25} = -38\overset{\circ}{.}6;\ [M]_D^{25} = -74\overset{\circ}{.}2$
0.0971 g acid in *ethyl acetate*, made up to 10.00 ml:
$\alpha_D^{20} = -0\overset{\circ}{.}397;\ [\alpha]_D^{20} = -40\overset{\circ}{.}9;\ [M]_D^{20} = -78\overset{\circ}{.}6$
0.1007 g acid in *alcohol*, made up to 10.00 ml:
$\alpha_D^{20} = -0\overset{\circ}{.}333;\ [\alpha]_D^{20} = -33\overset{\circ}{.}1;\ [M]_D^{20} = -63\overset{\circ}{.}6$
0.1088 g acid in *chloroform*, made up to 10.00 ml:
$\alpha_D^{20} = -0\overset{\circ}{.}261;\ [\alpha]_D^{20} = -24\overset{\circ}{.}0;\ [M]_D^{20} = -46\overset{\circ}{.}1$

**Quinidine salt of (—)-S-methylmercaptoethylsuccinic acid.** The salt crystallized from dilute acetone in colourless needles containing two moles of water of crystallization.

*Analysis*: 0.4187 g salt: loss in weight over $P_2O_5$ 0.0174 g
$C_7H_{12}O_4S,\ 2C_{20}H_{24}O_2N_2,\ 2H_2O$ calc. $H_2O$ 4.11
found » 4.16
0.0968 g dry salt: 5.590 ml $N_2$ at $18\overset{\circ}{.}2$ and 746.9 mm
$C_7H_{12}O_4S,\ 2C_{20}H_{24}O_2N_2$ calc. N 6.66
found » 6.65

## Thermal analysis

Contact preparates were studied according to KOFLER (8) using an "Original Kofler Heiztisch" and a "Heiztischmikroskop RCH" from Optische Werke Reichert. The thermal data are found in table 1.

Melting point diagrams were determined by RHEINBOLDT's method (12), using an apparatus described by FRIEDRICHS (13). The melting point data are found in tables 2—4.

*Table 2.*

(+)- and (—)-S-propylmercaptosuccinic acid.

| % (+)-acid | 50.0 | 56.5 | 60.6 | 65.1 | 69.3 | 75.0 | 80.9 | 85.3 | 90.4 | 100.0 |
|---|---|---|---|---|---|---|---|---|---|---|
| M. p. | 119°.5 | 118° | 117° | 116° | 114°.5 | 112° | 110°.5 | 113° | 115°.5 | 120° |

*Table 3.*

(+)- and (—)-S-methylmercaptoethylsuccinic acid.

| % (+)-acid | 50.0 | 60.0 | 69.9 | 80.1 | 85.1 | 87.5 | 90.2 | 95.2 | 100.0 |
|---|---|---|---|---|---|---|---|---|---|
| M. p. | 93° | 92°.5 | 90°.5 | 86° | 82° | 81° | 82° | 84° | 86° |

*Table 4.*

(+)-S-Propylmercaptosuccinic acid and
(—)-S-methylmercaptoethylsuccinic acid.

| % (+)-acid | 0.0 | 5.0 | 10.0 | 15.0 | 20.2 | 25.0 | 30.3 | 40.0 | 50.0 | 60.1 | 70.0 | 80.0 | 90.2 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M. p. | 85° | 83°.5 | 83° | 90° | 95° | 98° | 100° | 102° | 102°.5 | 101°.5 | 104° | 110° | 116° | 120° |

The author takes this opportunity of expressing his gratitude to Professor ARNE FREDGA for his valuable advice and help. The author is also indebted to Miss EVELYNE BUSCARLET for assistence with some of the melting point diagrams. The expenses involved in this investigation have been defrayed by a grant from the Nobel Fund of the Royal Swedish Academy of Sciences (Kemiska prisgruppens särskilda fond).

## SUMMARY

S-Propylmercaptosuccinic acid and S-methylmercaptoethylsuccinic acid were synthesized and resolved into optical antipodes.

The *racem*-forms were shown to be true racemic compounds.

It was found that S-propylmercaptosuccinic acid and S-methylmercaptoethylsuccinic acid with opposite directions of rotation form a quasi-racemic compound and from that it was concluded that the acids with the same direction of rotation have also the same configuration. S-Methylmercaptoethylsuccinic acid was correlated sterically to the glyceraldehyde system.

Laboratory of Organic Chemistry, University of Uppsala, January 1951.

REFERENCES: (1) **J. Timmermanns**, Rec. tr. chim. Pays-Bas [4] *48*, 890 (1929). — (2) **A. Fredga**, "The Svedberg 1884 30/8 1944", 261 (Uppsala 1944). — (3) **M. Matell**, To be published. — (4) **H. Lettré**, Z. angew. Chem. *50*, 581 (1937). — (5) **A. Fredga**, Arkiv Kemi, Mineral. Geol. *15 B*, N:o 23 (1942). — (6) ——, Ibid. *14B*, N:o 12 (1940). — (7) ——, Ibid. *14B*, N:o 15 (1940). — (8) **L.** and **A. Kofler**, Mikroskopische Methoden, 115 (Innsbruck 1948). — (9) **C. A. Bischoff**, Lieb. Ann. *214*, 38 (1882). — (10) Org. Syntheses, Coll. Vol. II, 136 (1947). — (11) **A. Fredga** and **U. Sahlberg**, Arkiv Kemi, Mineral. Geol. *18A*, N:o 16 (1944). — (12) **H. Rheinboldt**, J. prakt. Chem. [2] *111*, 242 (1925). — (13) **F. Friedrichs**, Z. angew. Chem. *34*, 61 (1921).

Tryckt den 12 april 1951

Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 18

Vorgelegt am 10. Januar 1951

# Zur Kenntnis der Reduktone. VIII

Von Hans von Euler und Hans Hasselquist

Mit 1 Figur im Text

## A. Trennungs-Versuche an Redukton und Redukton-Derivaten in Cis- und Trans-Formen

Nachdem es gelungen ist, aus dem bei etwa 140°–143° schmelzenden Redukton-Produkt A durch Behandlung mit 1-Methoxy-butadien ein bei 152°–153° schmelzendes Produkt II zu isolieren, das nicht mehr mit 1-Methoxy-butadien reagiert, wurde versucht, dieses Redukton II mittels Diazomethan zu methylieren.[1]

Es wurde eine Substanz HH 128 gewonnen, die bei 70°–71° schmilzt. Ihr Methoxyl-Gehalt stimmt angenähert, aber nicht genau mit demjenigen eines Monomethyläthers. Auch bei langer Methylierung mit einem Überschuss von Diazomethan wurde, wie früher mitgeteilt, keine Dimethylierung erzielt.

### Untersuchung des p-Amino-benzoesäure-reduktons bzgl. der Cis-Trans-Isomerie

675 mg Roh-redukton wurden in 50 ml Wasser gelöst und unter Umrühren zu einer Lösung von 1,05 g p-Amino-benzoesäure in 400 ml Wasser zugesetzt. Nach etwa 1 Min. fiel das Reaktionsprodukt in Form orangeroter Nadeln aus, die abfiltriert und mit Wasser gewaschen wurden (I). Aus dem Filtrat schieden sich noch weiter *gelbe Nadeln* (II) ab, die ebenfalls abfiltriert und gewaschen wurden. Auch die aus diesem Filtrat sich abscheidenden Fraktionen III und IV waren *gelb.*

I. 750 mg, Schmpt 290°
II. 58 mg, Schmpt 260–265°
III. 26 mg, Schmpt 253–255°

Je 5 mg dieser Fraktionen wurden mit 0,01 n. TR in Stickstoffatmosphäre titriert.

I. 4,45 ml TR verbrauchten äquiv. Gew. = 112
II. 3,70 ml TR » » » = 135
III. 1,90 ml TR » » » = 263
berechnet = 104

Ein Teil der Fraktion I, welche, der Titration zufolge, am reinsten erschien, wurde in Pyridin erwärmt, wodurch ein Anteil mit gelber Farbe in Lösung ging, während, auch bei längerem Kochen, rote Nadeln hinterblieben, welche abfiltriert und mit Pyridin gewaschen wurden. Sowohl diese roten Krystalle als das Filtrat verbrauchten TR. Beim Einengen liefert das Filtrat *gelbe* Nadeln. Die beiden Substanzen wurden durch Papier-chromatographie untersucht (Butanol-Wasser bei 20°C).

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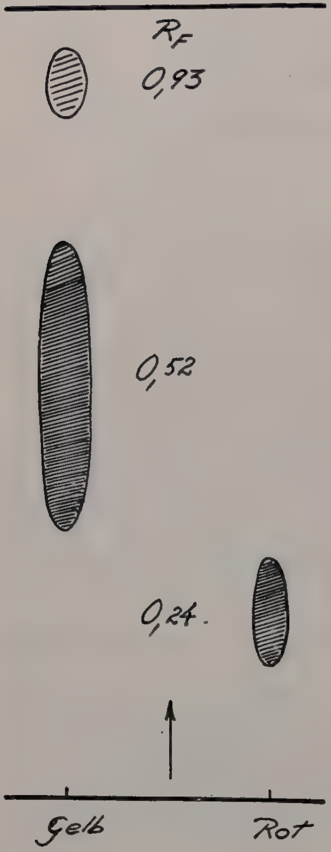
[1] Euler und H. Hasselquist, Dieses Arkiv 3, No 9 (1950).

$R_F$ rote Sbst. 0,24
$R_F$ gelbe Sbst. Hauptflecken = 0,52. Schwächerer Fleck 0,93
$R_F$ Redukton 0,64
Siehe Figur.



Unsere Papier-chromatographischen Versuche haben wir in einem rechteckigen Metallkasten ausgeführt, der 750 mm hoch war und eine Grundfläche von 400 mm × 200 mm besass. Die vordere senkrechte Wand (400 mm × 750 mm) war mit Fensterglas ersetzt. Die übrigen Wände waren mit aufgekittetem Milchglas bekleidet. Die obere Kasten-Seite war frei, und auf die plangeschliffenen Wandkanten konnte ein Glasdeckel dicht aufgesetzt werden.

Durch 2 in den senkrechten schmalen Wänden oben bezw. unten angebrachten Öffnungen konnte der Kasten während des Versuchs mit einem geeigneten Gas gefüllt werden.

Unterhalb des Glasdeckels konnte eine 595 mm lange Rinne von 15 mm Diameter eingesetzt werden. Von dieser mit dem Lösungsmittel gefüllten Rinne hing das Chromatographie-Papier (Munktell-OB-Papier) über einen auf gleicher Höhe befindlichen Glasstab senkrecht herunter.

### Darstellung von Mono-benzoyl-redukton (HH 130)

Mono-acetyl-redukton wurde von uns[1] aus dem Na-Salz des Reduktons dargestellt, das, in Benzol suspendiert, sich leicht mit Acetylchlorid umsetzt. In analoger Weise wurde das feinpulverisierte Na-Salz des Reduktons (500 mg) in 5 ml Benzol aufgeschlämmt, 15 Min. auf dem Wasserbad erwärmt. Die warme Lösung wurde sofort von dem gebildeten NaCl durch Filtration befreit und mit Aceton gewaschen.

Das Filtrat wurde im Vakuum bei 20° eingedunstet, worauf das Reaktionsprodukt in Form farbloser Prismen auskrystallisierte. Dieses mit Benzol gewaschene Rohprodukt, 400 mg, schmolz innerhalb 125°–138°. Nach zweimaligem Umkrystallisieren aus Äthylacetat schmolz die Sbst. konstant ohne Zersetzung bei 148°–149°.

HH 130 gibt mit $FeCl_3$ in alkoholischer Lösung eine kräftig braunrote Färbung, was die Anwesenheit einer Enolgruppe anzeigt. Die Sbst. ist leichtlöslich in Aceton, löslich in warmem Alkohol, Äthylacetat und Benzol, schwerlöslich in Wasser.

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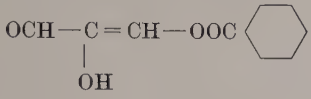
[1] EULER und H. HASSELQUIST, Rec. Trav. Chim. Pays-Bas 69, 402 (1950).

HH 130 verbraucht direkt nicht TR, aber bei Zusatz von wenig Alkali und Erwärmen tritt Hydrolyse ein, worauf TR reduziert wird.



*Analyse:*

| | | |
|---|---|---|
| Berechnet für $C_{10}H_8O_4$ | C = 62,50 | H = 4,20 |
| Gefunden | = 62,92 | = 4,37 |

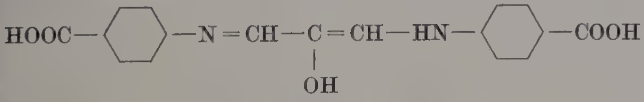
### Die Kondensation von p-Aminobenzoesäure und Redukton

Diese Reaktion haben wir bereits in vorhergehenden Mitteilungen besprochen.[1] Wir hatten in Übereinstimmung mit ANGIER et al. sowie mit BELL, COCKER und O'MEARA ein aus 1 Mol. p-ABS und 1 Mol. Redukton bestehendes Produkt gewonnen, während FORREST und WALKER[2] ein Kondensationsprodukt (Schmpt. 240°–250°) vom Komponenten-Verhältnis 2 p-ABS : 1 Redukton beschrieben. Bei der weiteren Verfolgung der Kondensation zwischen Redukton und p-ABS konnten wir eine *gelbe*, G, und eine *rote* Substanz, R, isolieren. Die rote Verbindung konnte wegen ihrer Schwerlöslichkeit in organischen Lösungsmitteln nicht umkrystallisiert werden. Sie löst sich zwar leicht in Alkali, dabei wird sie aber verändert (u. a. nimmt ihr TR-Verbrauch ab), so dass man sie mit Säuren nicht wieder fällen kann. Um die rote Substanz in analysenreiner Form zu erhalten wurde also folgendermassen verfahren:

Analysenreine p-ABS wurde vollständig in Wasser aufgelöst. Zu dieser analysenfiltrierten Lösung wurde eine ebenfalls analysenfiltrierte wässrige Lösung von Redukton gegeben. Nach etwa 1 Minute fiel zuerst die rote Substanz aus, die schnell abfiltriert wurde, bevor eine grössere Menge der gelben Substanz Zeit hatte auszufallen. Die rote Substanz wurde mit filtriertem dest. Wasser gewaschen und getrocknet. Die Krystalle wurden nun in wenig reinem Pyridin suspendiert. Man liess die Suspension 24 St. bei Zimmertemperatur stehen, wobei die gelbe Substanz vollständig in Lösung ging. Die ungelösten roten Krystalle wurden abfiltriert, mit Pyridin gewaschen, aufs neue in Pyridin suspendiert, nach 24 St. abfiltriert, mit Pyridin und reinem Benzol gewaschen, im Vakuum bei 90° getrocknet und analysiert.

*Analyse*: HH 133 (R):

| | |
|---|---|
| Berechnet für $C_{10}H_9O_4N$ | N = 8,60 % |
| Gefunden | = 9,11 % |



$E_{\text{Red}} = 163$ gef. 148, 151
$E_{\text{NaOH}} = 108{,}6$ gef. 96,5

Subst. G. Das rein gelbe Filtrat der Subst. R (HH 133) wurde unter kräftigem

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[1] EULER und H. HASSELQUIST, Rec. Trav. chim. Pays-Bas, 69, 402 (1950). — Dieses Arkiv 2 No 14 und 3 No 4 (1950).

[2] H. S. FORREST und J. WALKER, Chem. Soc. 1949 79 a. 2009.

Umschütteln mit Benzol gefällt; dabei fiel die gelbe Substanz G (das hauptsächliche Reaktionsprodukt) in Form kleiner Nadeln aus.

Subst. G ist leichtlöslich in Pyridin, schwerlöslich in allen übrigen üblichen organischen Lösungsmitteln.

Titration mit 0,01-n. TR.

Subst. G: 5 mg verbrauchten 4,3 ml TR. $E_{\text{gef}} = 116$, $E_{\text{ber}} = 104$.

Subst. R (HH 133): $E_{\text{gef}} = 148$, $E_{\text{ber}} = 163$.

### Acetylierung der gelben Subst. G. — Subst. HH 132

1,9 g der gelben Subst. wurden mit 3 ml Essigsäureanhydrid versetzt und das Gemisch wurde vorsichtig bis zum Siedepunkt erhitzt, wobei die Subst. nach etwa 1 Min. mit braunroter Farbe in Lösung ging. Nach Abkühlen auf Zimmertemperatur wurde die Lösung unter Umrühren mit Benzol versetzt wodurch das Reaktionsprodukt als schmutzigbraunes Pulver ausfiel, das abfiltriert und mit Benzol gewaschen wurde. Ausbeute 1,5 g.

Bei der Schmelzpunkts-Bestimmung sintert die Subst. bei 215 unter Dunkelbraunfärbung, schmilzt aber nicht unter 290°.

Durch zweimaliges Umkrystallisieren aus Alkohol erhält man mikroskopische, schwach gefärbte Prismen, die bei schnellem Erwärmen bei 227° ohne Zersetzung schmelzen. Die Substanz ist ziemlich leicht löslich in Methanol, Äthanol, Aceton, schwerlöslich in Benzol, Petroleumäther und Wasser. Sie verbraucht kein TR.

*Analyse:*

Berechnet für $C_{12}H_{11}O_5N$ N = 5,62 % Gef. N = 5,46 %

### Acetylierung der roten Substanz HH 133. — Subst. HH 134

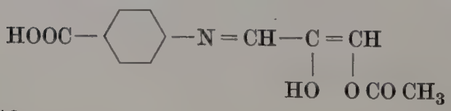
200 mg der roten Subst. HH 133 wurden mit 3 ml Essigsäureanhydrid versetzt. Bei 30 Min. langem Stehen auf dem Wasserbad ging die rote Subst. mit schwach brauner Farbe in Lösung. Nach Abkühlen wurde zu der Lösung etwas Benzol zugefügt und die Mischung wurde mit Petroleumäther gefällt, wodurch das Acetylderivat als schwach gelbes Öl ausfiel. Dasselbe wurde in wenig abs. Alkohol gelöst, aufs neue mit Petroleumäther gefällt und dieses Verfahren dreimal wiederholt, ohne dass das Öl krystallisierte.

Erst nach 3-tägigem Stehen im Kühlschrank bildeten sich aus dem Öl kleine farblose Prismen, die unter Braunfärbung bei 198°–203° schmolzen. Die Prismen wurden noch einmal aus absolutem Alkohol und Petroleumäther umkrystallisiert, wodurch der Schmpt. 204°–205° erhalten wurde. Die Ausbeute war infolge der vielen Umfällungen gering (15 mg).

Die Subst. HH 134 ist identisch mit Subst. HH 132. Beim Acetylieren von HH 132 scheidet p-ABS aus.

*Analyse:*

Berechnet für $C_{12}H_{11}O_5N$ N = 5,62 %
Gefunden 5,60 %



## B. Reduktionswirkungen in wachsenden Bakterien

Wachsende Bakterien enthalten teils solche Stoffe, welche — anscheinend auch ohne Mitwirkung eines Katalysators — Wasserstoff direkt an einen Wasserstoffacceptor (Farbstoff; Flavin) abgeben, teils Redoxasen, also Enzymsysteme, welche, in der Regel unter Vermittlung der Cozymase, ein enzymatisches Acceptor-System und gleichzeitig ein Donator-System katalysieren[1]. Zu diesen Systemen sind auch die $C_4$-Dicarbonsäuren (Szent-Györgyi) besonders auch Citronensäure (Martius u. Knoop) zu zählen, welche an das Diaphorase-Cytochrom-System angeschlossen sind.

Das Auftreten reduzierender Systeme in Mikroorganismen ist mit verschiedenen Phasen des Stoffwechsels und somit des Wachstums verknüpft. Im einzelnen sind diese Zusammenhänge zwischen Wachstum und oxydoreduktivem Stoffwechsel noch unbekannt.[2]

In den wachsenden Bakterien tritt nach O'Meara[3] und Cocker auch Redukton auf und es liegt nahe, die Bedingungen (Nährmedium, Acidität) aufzusuchen, von welchen die Redukton-Bildung begünstigt wird.

Mit den üblichen Testreagenzien des Reduktons, Dichlorphenolindophenol und o-Dinitrobenzol liessen sich in den hier untersuchten wachsenden Bakterien, z. B. *B. aerogenes* nur geringe Effekte nachweisen.

In manchen Fällen sind Tetrazoliumsalze — wir verdanken ein Präparat Herrn Professor Dr. P. Karrer — brauchbare Testsubstanzen für Reduktone in alkalischer Lösung. Bekannt ist, dass z. B. Ascorbinsäure in alkalischer Lösung mit Tetrazoliumsalzen unter Rotfärbung (Formazanbildung) reagiert. Auch an aliphatischen Oxysäuren fanden wir die gleiche Reaktion. Unter den aromatischen Reduktonen (Brenzcatechinderivaten) mögen Adrenalin und Arterenol genannt sein.

Kuhn und Jerchel[4] haben eine systematische Untersuchung über das „anscheinende Reduktionspotential" einer Reihe von Farbstoffen angestellt.

Triose-Redukton färbt sich mit Triphenyl-tetrazolium-chlorid intensiv rot erst in alkalischer Lösung. Ein Versuch mit verdünntem Triphenyl-tetrazolium-chlorid ergab folgendes:

| | pH | |
|---|---|---|
| 2 ml Redukton-Lös. (1 mg/ml) + | 5,15 | beginnende schwache Rosafärbung |
| 1 ml 0,1%ige Tetrazolium-chlorid-Lös. | 6,20 | schwache Rosafärbung und rotviolette Fluorescens |
| | 10,70 | dunkelrote Färbung und Fällung |

Über das Verhalten unserer Bakterien gegen Methylenblau und gegen TR haben wir im Thunberg-Rohr einige orientierende Versuche angestellt.[5]

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[1] Euler, E. Adler und H. Hellström, Z. physiol. Chem. 241, 239 (1936).

[2] Triose-Redukton ist — im Gegensatz zu Ascorbinsäure — ein Inhibitor des Wachstums von Mikroorganismen (Hasselquist; Bánhidi; Ander) Experientia, 5, 81 (1949), Karrer Festschrift. — Dieses Arkiv 3 No 5 (1951).

[3] O'Meara et al., Nature 154, 796 (1944).

[4] R. Kuhn und D. Jerchel, Chem. Ber. 74, 949 (1941).

[5] An der Ausführung dieser Versuche waren Herr Ing. Rogne Ander und Frl. Marianne Freyne beteiligt.

## Methodik

Im allgemeinen wurde nach der Thunberg-Ahlgren-schen Methodik gearbeitet. Für die Bakterien kam folgende Nährlösung zur Verwendung:

0,3 % Beef-extract
0,5 % Bactopepton
0,5 % NaCl
0,1–1 % Substrat
} Mischung A

Zu 5 ml Mischung A wurden 1 ml 0,3-m. $PO_4$-pufferlös.zugesetzt.

Nach 4 resp. 6 Stunden wurden zu jedem Versuch 30 ml herausgenommen und centrifugiert. Das Centrifugat wurde:

a. zur Trockengewicht-Best.: mit Wasser, Aceton und Äther gewaschen,
b. zur Tillmans-Titration: mit etwas Wasser und mit 1 Tr. Eisessig versetzt,
c. zu Thunberg-Vers. pH 6,8: in 1 ml 0,3-m. Phosphatpufferlös. suspendiert,
d. zu » » pH 5: in 1 ml Wasser + 1 Tr. Eisessig » .

## Versuche

Versuchsreihe I–III. Substrat: Glucose.

| Vers. Nr | Dauer d. Wachstums Stunden | Trocken-gewicht mg/30 ml | Titr. mit 0,001 n-TR pH 5 | Thunberg-Versuche | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0,25 ml Aussen: | 0,01-n. TR 1 ml | 0,25 ml 0,01 n. Mb | |
| | | | | | | suspend. | Centrifugat |
| | | | | pH 5 | pH 6,8 | pH 5 | pH 6,8 |
| III Ohne Glucose | 4 | 1,7 | 0,05 ml | 0 | 0 | 0 | 0 |
| | 6 | 1,0 | 0,05 ml | 0 | 0 | 0 | 0 |
| I 0,1 % Glucose | 4 | 2,9 | 0,1 | 0 | 12 Min. | 0 | 4,15 Min |
| | 6 | 5,8 | 0,1 | 0 | 7,3 Min. | 0 | 3 Min |
| II 1 % Glucose | 4 | 3,2 | 0,05 | — | — | 0 | 3,1 Min |
| | 6 | 3,6 | 0,1 | — | — | 0 | 1 Min |

0 bedeutet in dieser und den folgenden Tabellen: Keine Entfärbung.

Zufolge den Vers.-Reihen I–III war in den untersuchten Bakterien-Suspensionen praktisch kein Endiol nachweisbar. Ein gewisser Effekt bei Zusatz von Glucose bzgl. der Entfärbung des Methylenblaues bei pH 6,8 rührt nicht vom Endiol her sondern vermutlich von Cystein oder dergl., was nicht hervortritt ohne Zusatz von Glucose zur Nährlösung.

Der Umstand, dass beim Versuch V keine Entfärbung eintritt, wie bei Na-Succinat, dürfte darauf beruhen, dass die zur Citrico-Dehydrase erforderliche Cozymase im Versuch V in unzureichender Menge vorhanden war. Damit hängt auch das geringere Bakterien-Wachstum (die geringere Zunahme des Trockengewichts) zusammen. Auch in unseren mit Succinat und mit Citrat vorbehandelten

Versuchsreihe IV und V. Substrat: Na-Succinat.

| Vers. Nr | Dauer d. Wachstums Stunden | Trockengewicht mg/30 ml | Titr. mit 0,001-n. TR pH 5 | Thunberg-Versuche | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0,25 ml 0,01-n. TR | | 0,25 ml 0,01-n. Mb | |
| | | | | pH 5 | pH 6,8 | pH 5 | pH 6,8 |
| IV<br>0,1 %<br>Na-Succinat | 4 | 1,8 | 0,1 | 0 | 12 Min | 0 | 11,5 Min |
| | 6 | 4,2 | 0,1 | 0 | 6 Min | 0 | 6 Min |
| V<br>0,1 %<br>Na-Citrat | 4 | 1,2 | 0,05 | 0 | 0 | 0 | 0 |
| | 6 | 1,6 | 0,05 | 0 | 0 | 0 | 0 |

Bakterien-Suspensionen (R. ANDER) fanden wir kein Anzeichen für die Anwesenheit eines Endiols, wie nach O'MEARA und COCKER zu erwarten wäre. Dieses Ergebnis entspricht den von FR. WEYGAND[1] mitgeteilten, an verschiedenen Bakterien-Stämmen gewonnenen Resultaten.

Stockholm, Universität, Vitamin-Institut.

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[1] FR. WEYGAND, Dieses Arkiv, 3 No 2 (1951).

Tryckt den 20 april 1951

Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 19

Communicated 14 February 1951 by ARNE FREDGA

# The hydrolysis of β-phenyl-β-cyanopropionic acid

By SIGVARD WIDEQVIST

As reported by the author in previous publications (1), (2), (3) β-phenyl-β-cyanopropionic acid is spontaneously hydrolyzed to phenylsuccinamic acid in water solution even at room temperature:

$$C_6H_5—CH(CN)—CH_2—COOH + H_2O \rightarrow C_6H_5—CH(CONH_2)—CH_2—COOH.$$

The amic acid is further hydrolyzed to the acid ammonium salt of phenylsuccinic acid:

$$C_6H_5—CH(CONH_2)—CH_2—COOH + H_2O \rightarrow C_6H_5—CH(COONH_4)—CH_2—COOH.$$

In alkaline solution, however, β-phenyl-β-cyanopropionic acid is difficult to hydrolyze, as found by BREDT and KALLEN (4). The anion of the cyano-acid seems to be very resistant to hydrolysis.

We may thus assume that it is the undissociated acid which hydrolyzes. We may further assume that the hydrolysis is partly uncatalyzed and partly catalyzed by hydrogen ions. With these assumptions we obtain the following differential equation for the velocity of the hydrolysis:

$$dx/dt = (k_1 + k_2[H^+])(1 - \alpha_1)(c - x) \qquad (1)$$

where $c$ is the concentration of the cyano-acid at zero time, $(c - x)$ the concentration at time $t$, $\alpha_1$ the degree of ionization and $k_1$ and $k_2$ the rate constants for the uncatalyzed and catalyzed reactions. Upon setting $(k_1 + k_2[H^+]) = k_N$ we get

$$dx/dt = k_N(1 - \alpha_1)(c - x). \qquad (1\,a)$$

It is very likely that the hydrolysis of phenylsuccinamic acid takes place according the same scheme. The mechanism of this reaction is, however, quite unimportant for the treatment of the hydrolysis of the cyano-acid. We may thus assume that the differential equation for the hydrolysis of the amic acid is

$$dy/dt = k_A(x - y) \qquad (2)$$

where $(x - y)$ is the concentration of the amic acid at time $t$, $y$ the concentration of the ammonium hydrogen phenylsuccinate and $k_A$ the rate constant which may also include hydrogen ion concentration and the degree of ionization of the amic acid ($\alpha_2$).

Let us denote the cyano-acid by HN, the corresponding amic acid by HA and the phenylsuccinic acid by $H_2B$. When all the three acids are present in a water solution, we get the following equilibria:

$$\begin{array}{lcl}
\mathrm{HN} & \rightleftharpoons \mathrm{H}^+ + & \mathrm{N}^- \\
(1-\alpha_1)(c-x) & h & \alpha_1(c-x) \\
\mathrm{HA} & \rightleftharpoons \mathrm{H}^+ + & \mathrm{A}^- \\
(1-\alpha_2)(x-y) & h & \alpha_2(x-y) \\
\mathrm{H_2A} & \rightleftharpoons \mathrm{H}^+ + & \mathrm{HB}^- \\
(1-\alpha_3)\,y & h & \alpha_3\,y
\end{array}$$

where $h$ is the concentration of hydrogen ions, $\alpha_2$ and $\alpha_3$ the degrees of ionization of resp. phenylsuccinamic acid and phenylsuccinic acid. The second-stage dissociation of the succinic acid may be neglected. Thus we have the following relations:

$$[\mathrm{HN}] + [\mathrm{N}^-] = c - x;\ [\mathrm{HA}] + [\mathrm{A}^-] = x - y;\ [\mathrm{H_2B}] + [\mathrm{HB}^-] = y;\ [\mathrm{NH}_4^+] = y;$$
$$[\mathrm{H}^+] = h;\ [\mathrm{N}^-] = \alpha_1(c-x);\ [\mathrm{A}^-] = \alpha_2(x-y);\ [\mathrm{HB}^-] = \alpha_3\,y.$$

According to the conditions for electrical neutrality we get

$$[\mathrm{H}^+] + [\mathrm{NH}_4^+] = [\mathrm{N}^-] + [\mathrm{A}^-] + [\mathrm{HB}^-]$$

or

$$h + y = \alpha_1(c-x) + a_2(x-y) + \alpha_3\,y. \tag{3}$$

The rate of hydrolysis may be determined by conductivity measurements. The following additional symbols are introduced:

$K_1, K_2, K_3$ = dissociation constants for HN, HA, and $H_2B$.

$\Lambda_{\mathrm{N}}, \Lambda_{\mathrm{A}}, \Lambda_{\mathrm{HB}}, \Lambda_{\mathrm{H}}, \Lambda_{\mathrm{Am}}$ = ion mobilities for $N^-$, $A^-$, $HB^-$, $H^+$, and $NH_4^+$.

$\varkappa$ = specific conductivity of the solution at time $t$.

$\varkappa_0$ = specific conductivity of the solution at time 0.

$t$ = time in minutes.

For the specific conductivity of the solution we get the expression

$$1000\,\varkappa = h\,\Lambda_{\mathrm{H}} + y\,\Lambda_{\mathrm{Am}} + (c-x)\,\alpha_1\,\Lambda_{\mathrm{N}} + (x-y)\,\alpha_2\,\Lambda_{\mathrm{A}} + y\,\alpha_3\,\Lambda_{\mathrm{HB}}. \tag{4}$$

Further we have

$$h\,\alpha_1/(1-\alpha_1) = K_1\ (5);\quad h\,\alpha_2/(1-\alpha_2) = K_2\ (6);\quad h\,\alpha_3/(1-\alpha_3) = K_3. \tag{7}$$

We shall now find the relation between the reaction velocity, $dx/dt$, and the change in the conductivity, $d\varkappa/dt$, at time $t = 0$. The calculations are largely simplified by assuming that the mobilities of the anions are approximately the same, namely $\Lambda_{\mathrm{N}}$. Then we get

$$1000\,\varkappa = h\,\Lambda_{\mathrm{H}} + y\,\Lambda_{\mathrm{Am}} + [(c-x)\,\alpha_1 + (x-y)\,\alpha_2 + y\,\alpha_3]\,\Lambda_{\mathrm{N}} \tag{5 a}$$

Combining equ. (3) and (5 a) we get

$$1000\,\varkappa = h\,\Lambda_{\mathrm{H}} + y\,\Lambda_{\mathrm{Am}} + (h+y)\,\Lambda_{\mathrm{N}} \tag{8}$$

or

$$1000\,\varkappa = h\,\Lambda_{\mathrm{HN}} + y\,\Lambda_{\mathrm{AmN}} \tag{8 a}$$

where

$$\Lambda_{\mathrm{HN}} = \Lambda_{\mathrm{H}} + \Lambda_{\mathrm{N}} \quad \text{and} \quad \Lambda_{\mathrm{AmN}} = \Lambda_{\mathrm{Am}} + \Lambda_{\mathrm{N}}.$$

On differentiating equ. (8 a) we find

$$1000\,d\varkappa = \Lambda_{\mathrm{HN}}\,dh + \Lambda_{\mathrm{AmN}}\,dy. \tag{9}$$

From equ. (5), (6) and (7) we obtain

$$\alpha_1 = K_1/(h+K_1); \;\; \alpha_2 = K_2/(h+K_2); \;\; \alpha_3 = K_3/(h+K_3).$$

When these values of $\alpha_1$, $\alpha_2$, and $\alpha_3$ are introduced into equ. (3) we have

$$h + y = (c-x)\,K_1/(h+K_1) + (x-y)\,K_2/(h+K_2) + y\,K_3/(h+K_3). \tag{10}$$

On differentiation we get

$$\begin{aligned} dh + dy = &-K_1\,(c-x)\,dh/(h+K_1)^2 - K_1\,dx/(h+K_1) - K_2\,(x-y)\,dh/(h+K_2)^2 + \\ &+ K_2\,(dx-dy)/(h+K_2) + K_3\,dy/(h+K_3) - K_3\,y\,dh/(h+K_3)^2. \end{aligned} \tag{11}$$

But according to equ. (1) and (2) we hawe

$$dx = k_{\mathrm{N}}\,(1-\alpha_1)\,(c-x)\,dt \quad \text{and} \quad dy = k_{\mathrm{A}}\,(x-y)\,dt.$$

Thus we get $x$, $y$, and $dy = 0$ at time $t = 0$. At $t = 0$ equ. (9) and (11) simplifie to

$$1000\,d\varkappa = \Lambda_{\mathrm{HN}}\,dh \tag{9 a}$$

and

$$dh = -K_1\,c\,dh/(h+K_1)^2 - K_1\,dx/(h+K_1) + K_2\,dx/(h+K_2). \tag{11 a}$$

Equ. (11 a) may also be written

$$[1 + K_1^2\,c/K_1\,(h+K_1)^2]\,dh = [K_2/(h+K_2) - K_1/(h+K_1)]\,dx.$$

Combining this expression with equ. (5) and (6) we obtain

$$(2-\alpha_1)\,dh = (\alpha_2-\alpha_1)\,dx. \tag{12}$$

From equ. (9 a) and (12) we get

$$1000\,d\varkappa = -\Lambda_{\mathrm{HN}}\,(\alpha_1 - \alpha_2)\,dx/(2-\alpha_1). \tag{13}$$

Combining equ. (13) with equ. (1 a) we obtain

$$-k_{\mathrm{N}} = \frac{1000\,(2-\alpha_1)\,d\varkappa/dt}{(1-\alpha_1)\,(\alpha_1-\alpha_2)\,c\,\Lambda_{\mathrm{HN}}}. \tag{14}$$

This expression is identical with that previously derived by the author (5) for the hydrolysis of α-thiocyanopropionic acid. This reaction, however, proceeds only one step and stops at the amic acid (S-aminoformylthiolactic acid). The velocity constant obtained with equ. (14) was identical with that obtained by FREDGA (6) from polarimetric measurements on optically active α-thiocyanopropionic acid.

Equ. (13) may be converted into a more convenient form in the following way. Combining equ. (5) and (6) and putting $K_1/K_2 = \varphi$ we get

$$1/\alpha_2 - 1 = (1/\alpha_1 - 1)\,K_1/K_2 = \varphi\,(1-\alpha_1)/\alpha_1.$$

From this we get

$$\alpha_2 = \alpha_1/(\varphi\,(1-\alpha_1) + \alpha_1)$$

and

$$\alpha_1 - \alpha_2 = \frac{\varphi\,(1-\alpha_1)\,\alpha_1 + \alpha_1^2 - \alpha_1}{\varphi\,(1-\alpha_1) + \alpha_1}.$$

After simplification this becomes

$$\alpha_1 - \alpha_2 = \frac{\alpha_1\,(1-\alpha_1)}{\varphi/(\varphi-1) - \alpha_1} = \frac{\alpha_1\,(1-\alpha_1)}{\psi - \alpha_1}$$

when $\psi = \varphi/(\varphi - 1)$. On introduction of this expression into equ. (14) we get:

$$k_{\mathrm{N}} = -\frac{1000\,(2-\alpha_1)\,(\psi-\alpha_1)}{(1-\alpha_1)^2\,\alpha_1\,c\,\Lambda_{\mathrm{HN}}}\cdot\frac{d\varkappa}{dt}. \tag{15}$$

But $\alpha_1 c\,\Lambda_{\mathrm{HN}} = 1000\,\varkappa_0$. Thus we get

$$k_{\mathrm{N}} = -\frac{(2-\alpha_1)\,(\psi-\alpha_1)}{(1-\alpha_1)^2\,\varkappa_0}\,\frac{d\varkappa}{dt} \tag{16}$$

where $\varkappa_0$ is the specific conductivity at time $t = 0$. For $k_{\mathrm{N}}$ we have the relation

$$k_{\mathrm{N}} = k_1 + k_2\,[\mathrm{H}^+].$$

At time $t = 0$ we have $[\mathrm{H}^+] = \alpha_1 c$. But $\alpha_1 c = 1000\,\varkappa_0/\Lambda_{\mathrm{HN}}$. By plotting $k_{\mathrm{N}}$ against $1000\,\varkappa_0/\Lambda_{\mathrm{HN}}$ we obtain a straight line with $k_1$ as intercept at $[\mathrm{H}^+] = 0$ and $k_2$ as slope.

## Experimental

β-Phenyl-β-cyanopropionic acid was prepared from ethyl phenylcyanoacetate and ethyl chloroacetate according to WIDEQVIST (2). M. p. 75°. The conductivity was measured for several concentrations at 25° C. As phenylsuccinamic acid is weaker than β-phenyl-β-cyanopropionic acid, the conductivity of the solution decreases with time. The conductivity was followed for about 400 minutes, and readings were taken every 30th minute. When $\varkappa$ was plotted against $t$ a straight line was obtained; $\varkappa_0$ and $d\varkappa/dt$ were determined by the method of least squares. The results of the measurements are shown in table I. The values of $\alpha_1$ and $h$ are calculated assuming that OSTWALD's dilution law is valid. For $\Lambda_{\mathrm{HN}}$ the value 369 was adopted. The dissociation constant of β-phenyl-β-cyanopropionic acid is $1.62\ .10^{-4}$ and of phenylsuccinamic acid $3.76\ .10^{-5}$. (WIDEQVIST (2).) From that we obtain $\varphi = 4.309$ and $\psi = 1.302$.

*Table I*

The hydrolysis of β-phenyl-β-cyanopropionic acid at 25° C.

| $c \cdot 10^3$ | $1000\ \varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha_1$ | $h \cdot 10^4$ | $k_{\mathrm{N}} \cdot 10^4$ |
|---|---|---|---|---|---|
| 1.298 | 0.14194 | 0.457 | 0.2959 | 3.847 | 1.11 |
| 4.768 | 0.29756 | 1.202 | 0.1691 | 8.064 | 1.21 |
| 4.916 | 0.30041 | 1.185 | 0.1656 | 8.141 | 1.18 |
| 7.710 | 0.38704 | 1.638 | 0.1360 | 10.49 | 1.23 |
| 9.668 | 0.43326 | 1.911 | 0.1215 | 11.74 | 1.27 |
| 15.64 | 0.56116 | 2.480 | 0.0972 | 15.20 | 1.24 |
| 19.71 | 0.62966 | 2.920 | 0.0866 | 17.06 | 1.29 |

From the values of $h$ and $k_{\mathrm{N}}$ in table I we may calculate the velocity constants $k_1$ and $k_2$ according to the relation

$$k_{\mathrm{N}} = k_1 + k_2 h.$$

The calculation is conveniently performed by the method of least squares which gives

$$k_1 = 1.10\ .10^{-4}$$

$$k_2 = 1.13\ .10^{-2}.$$

By means of the values of $k_1$ and $k_2$ we may recalculate $k_{\mathrm{N}}$ at different concentrations of hydrogen ions. In table II the calculated $k_{\mathrm{N}}$-values are compared with those experimentally found.

*Table II*

The velocity constant $k_{\mathrm{N}}$ at various conc. of hydrogen ions.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $h \cdot 10^4$, | 3.847 | 8.064 | 8.141 | 10.49 | 11.74 | 15.20 | 17.06 |
| $k_{\mathrm{N}} \cdot 10^4$, found | 1.11 | 1.21 | 1.18 | 1.23 | 1.27 | 1.24 | 1.29 |
| $k_{\mathrm{N}} \cdot 10^4$, calcd | 1.14 | 1.19 | 1.19 | 1.22 | 1.23 | 1.27 | 1.29 |

## Summary

β-Phenyl-β-cyanopropionic acid hydrolyzes spontaneously in water solution at room temperature yielding phenylsuccinamic acid. The hydrolysis is partly uncatalyzed and partly catalyzed by hydrogen ions, and it is the undissociated cyano-acid which hydrolyzes. The velocity constants for the uncatalyzed ($k_1 = 1.10 \cdot 10^{-4}$) and the catalyzed ($k_2 = 1.13 \cdot 10^{-2}$) reaction have been determined by conductivity measurements at 25° C.

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REFERENCES. (1) **Wideqvist,** Arkiv Kemi, Mineral. Geol. *14 B,* No. 19 (1940). — (2) ——, Svensk Kem. Tid. *54,* 34 (1942). — (3) ——, Sjätte Nordiska Kemistmötets Förh. Lund 1948. — (4) **Bredt** and **Kallen,** Ann. *293,* 343 (1896). — (5) **Wideqvist,** Svensk Kem. Tid. *55,* 284 (1943). — (6) **Fredga,** J. prakt. Chem. [2] *123,* 110 (1929).

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# X-ray investigations on $BiNbO_4$, $BiTaO_4$ and $BiSbO_4$

By BENGT AURIVILLIUS

With 2 figures in the text

From the chemical properties and radii of Sb (III) and Bi (III) on the one hand and Sb(V), Nb(V) and Ta(V) on the other, the crystal structures of $BiNbO_4$, $BiTaO_4$ and $BiSbO_4$ might be expected to be closely related to that of stibiotantalite ($Sb(III)Ta(V)O_4$) (For the structure of stibiotantalite see (1)). In fact powder photographs indicate that the mineral bismutotantalite (mainly $BiTaO_4$) is isomorphous with stibiotantalite (2). The present investigation deals with the synthetical compounds $BiNbO_4$, $BiTaO_4$ and $BiSbO_4$. The structure of $BiSbO_4$ has been determined; as expected it resembles that of stibiotantalite. In the case of $BiNbO_4$ and $BiTaO_4$ only the unit cell dimensions have been determined. These, however, proved to be related to those of stibiotantalite.

## Unit cell dimensions of $BiNbO_4$ and $BiTaO_4$

The samples were prepared by heating calculated amounts of $Bi_2O_3$ and $Nb_2O_5$ or $Ta_2O_5$ in air in platinum or porcelain crucibles to 1100°—1200° C. Powder photographs showed that the samples were not isomorphous with stibiotantalite and thus probably not with bismutotantalite either. Since this might be due to $Nb_2O_5$ or $Ta_2O_5$ having lost $O_2$ during heating, the experiments were repeated in a stream of $O_2$ under which circumstances a loss of $O_2$ seemed improbable (see (3)). However, the same powder photographs were obtained as before. Crystals for single crystal work were taken from melts with (50 at % $Bi_2O_3$ + 50 at % $Nb_2O_5$) and (60 at % $Bi_2O_3$ + 40 at % $Ta_2O_5$). Zero and first order Weissenberg photographs around (010) and (001) were taken. For both phases the crystals were found to be twinned in such a way that rows in the Weissenberg photographs which would normally contain $h0l$ or $hk0$ actually contained ($h0l + h0\bar{l}$) and ($hk0 + h\bar{k}0$).

The slanting lines $0k0$ and $00l$ but not $h00$ were doubled. No attempt was made to divide the twins into single components. Since the $h0l$ and $hk0$ spots differed from the corresponding $h0\bar{l}$ and $h\bar{k}0$ in $\sin^2\theta$ and intensity the symmetry was assumed to be triclinic. Patterson projections P($xyp$) and P($xpz$) made for $BiNbO_4$ and $BiTaO_4$ indicated that these phases are isomorphous. The reciprocal cell elements $a^*$, $b^*$, $c^*$, $\beta^*$, $\gamma^*$ were calculated from the zero order Weissenberg photographs. For the determination of the angle $\alpha^*$ either the lengths of the axes $b$ and $c$—as found from the rotation photographs—could be used or the calculations could be based on the first order photographs. The latter alternative was chosen, and from the difference in $\sin^2\theta$ of some spots $h1l$ and $h\bar{1}l$ the conclusion was drawn that $\cos\alpha^* \approx 0$, $\alpha^* \approx 90°$. In this way the following values were arrived at:

| | $a$ | $b$ | $c$ | $\alpha$ | $\beta$ | $\gamma$ | $V(\text{Å})^3$ |
|---|---|---|---|---|---|---|---|
| $BiNbO_4$ .................. | $7.7_1$ | $5.5_5$ | $7.9_7$ (Å) | 89° | 77° | 87° | 332 |
| $BiTaO_4$ .................. | $7.6_9$ | $5.6_0$ | $7.7_8$ | 89° | 77° | 86° | 325 |

From the rotation photographs the lengths of the $b$ and $c$ axes were found to be $5.6_1$ and $7.9_7$ Å for $BiNbO_4$ and $5.6_1$ and $7.7_8$ Å for $BiTaO_4$. The inaccuracy of the above figures is probably rather large, amounting to 1—2 % in the lengths of the axes and 3—6 % in the cell volumes. With the above unit cells no systematic extinctions were found. Considering the inaccuracy of the cell volumes the observed densities 7.5 and 9.0 agree fairly well with the assumption of 4 $BiNbO_4(BiTaO_4)$/unit cell (calculated densities 7.3 and 9.3). $SbTaO_4$ has previously been shown to contain 4 formula units/unit cell and the cell volume has been determined as 323 Å$^3$.* The axes of $SbTaO_4$ (orthorhombic symmetry) are: $a = 4.927$ $b = 5.554$ and $c = 11.81$ Å. These figures might be compared with the cell edges of $BiNbO_4$ written in the B centered form: $a = 9.7_8$ $(2 \cdot 4.89)$ $b = 5.5_4$, $c = 12.2_3$ Å, $\alpha \approx \beta \approx \gamma = 88°$

## $BiSbO_4$ phase

Single crystals were prepared from a mixture of $Bi_2O_3$ and $Sb_2O_3$ with the original ratio $Bi_2O_3/Sb_2O_3 = 3:2$ (60 at % $Bi_2O_3$) which was heated in air in an Au crucible to about 1000° C. Powder and Weissenberg photographs were taken. Some of the lines in the powder photographs could be explained with the aid of the unit cell found from the Weissenberg photographs. To get a pure phase, mixtures of $Bi_2O_3$ and $Sb_2O_3$ in the mol ratio 1 : 1 were heated in gold or platinum crucibles either directly to 900° C for a couple of days or first to 600° C for a couple of days and then to 900° C for a week. Except for a few very weak lines (see Table 1) all lines of these photographs could be explained by the unit cell given below. The positions of the lines were the same as those found for the 60 % sample. Analysis for surplus oxygen (distillation of the sample with HCl + KI and titrating the liberated I) gave 3.95 and 4.06 % O for the above samples whereas the calculated value for $BiSbO_4$ is 4.06 %. It thus seemed probable that the formula is $BiSbO_4$. From the method of preparation it seemed probable that the Sb atoms are in the pentavalent state.

It might be mentioned that when $Bi_2O_3$ is rapidly heated with an excess of $Sb_2O_3$ to 900° C in porcelain, gold or platinum crucibles, samples are obtained which give the same powder photographs as the above 50 % sample except for small shifts in $\sin^2 \theta$, leading to slightly different unit cell dimensions. However, when samples of the same original composition are heated first to 600° C and then to 900° C in platinum crucibles the powder photographs give the lines of the 50 % sample with unaltered positions and in addition the lines of $Sb_2O_4$. Explanations of this behaviour might be either that atoms from the crucibles replace Sb or Bi in the $BiSbO_4$ lattice or that Bi is replaced by Sb (III) in the presence of excess Sb. If the latter is assumed, we must also assume that $Sb_2O_4$ reacts very slowly with $BiSbO_4$.

The crystals were flat needles or plates of irregular forms. Some of the needles were striated along the line (100). In the striated crystals the $b$ axis was perpendicular to the plane of the needle, whereas the needle axis was found to lie along the line (601). For the plates the largest face was parallel to the

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* referred to $\mathrm{Cr}K_\alpha = 2.2909$ Å

*Table 1*

Powder photographs of $BiSbO_4$. Cr K radiation. Figures in brackets indicate that the corresponding reflexions coincide with a $\beta$ reflexion. Observed and calculated $\sin^2\theta$ are given up to $\sin^2\theta = 0.95$

| $h\,k\,l$ | $10^4$ $\sin^2\theta$ calc | $10^4$ $\sin^2\theta$ obs. | $I$ obs |
|---|---|---|---|
| (1 1 0) | 1005 | 1008 | (m) |
| 1 1 $\bar{2}$ | 1235 | 1225 | st |
| 1 1 2 | 1557 | 1552 | st |
| 0 0 4 | 1565 | | |
| 2 0 0 | 1823 | 1823 | m |
| 0 2 0 | 2197 | 2199 | m |
| 1 1 $\bar{4}$ | 2246 | 2238 | w |
| 2 0 2 | 2535 | 2530 | w |
| 0 2 2 | 2587 | 2586 | w |
| 2 0 $\bar{4}$ | 2742 | 2726 | m |
| 1 1 4 | 2890 | 2883 | vw |
| 0 1 5 | 2987 | 2982 | vw |
| 0 0 6 | 3522 | 3510 | vvw |
| — | — | 3576 | vvw |
| 0 2 4 | 3762 | 3753 | st |
| 2 1 $\bar{5}$ | 4006 | 4019 | vst |
| 2 2 0 | 4020 | | |
| 2 0 4 | 4030 | | |
| 1 1 $\bar{6}$ | 4038 | | |
| (2 2 $\bar{2}$) | 4088 | 4088 | (w) |
| 3 1 $\bar{2}$ | 4558 | 4557 | m |
| (3 1 0) | 4651 | 4650 | (w) |
| 2 2 $\bar{4}$ | 4939 | 4933 | m |
| 1 1 6 | 5004 | 4989 | m |
| 1 3 0 | 5400 | 5398 | w |
| 3 1 2 | 5524 | 5535 | $m^+$ |
| 2 1 5 | 5616 | 5628 | st |
| 1 3 $\bar{2}$ | 5629 | | |
| 0 2 6 | 5713 | 5711 | vw |
| 1 3 2 | 5951 | 5955 | st |
| 2 1 $\bar{7}$ | 6024 | 6037 | vw |
| 2 2 4 | 6227 | 6233 | st |
| 0 0 8 | 6251 | | |
| 2 2 $\bar{6}$ | 6569 | 6573 | w |
| 2 3 $\bar{1}$ | 6705 | 6714 | m |
| 3 1 $\bar{6}$ | 6717 | | |
| 2 0 $\bar{8}$ | 6785 | 6774 | m |
| (1 3 4) | 7285 | 7293 | (m) |
| (4 0 0) | 7296 | | |
| 4 0 4 | 7567 | 7569 | m |
| (4 1 $\bar{3}$) | 7757 | 7754 | (w) |
| (4 1 $\bar{1}$) | 7862 | 7865 | (w) |
| (1 1 8) | 7900 | 7904 | (w) |
| (3 2 3) | 7904 | | |
| (2 3 3) | 8129 | 8112 | (w) |
| 1 3 $\bar{6}$ | 8433 | 8443 | vst |
| 0 2 8 | 8448 | | |
| 2 2 6 | 8503 | 8503 | vw |
| 0 4 0 | 8789 | 8787 | st |
| 3 3 $\bar{2}$ | 8953 | 8967 | st |
| 3 1 $\bar{4}$ | 8969 | | |
| 2 2 $\bar{8}$ | 8982 | | |
| 0 4 2 | 9179 | 9181 | w |
| 2 0 8 | 9363 | 9352 | $m^+$ |
| 3 2 $\bar{7}$ | 9388 | 9392 | m |
| 1 3 6 | 9399 | | |
| 1 4 1 | 9424 | | |
| 4 2 0 | 9490 | 9496 | m |

plane (001). The Weissenberg photographs taken registered: $0kl$, $1kl$, $2kl$; $h0l$, $h\bar{1}l$, $h2l$; $hk0$ $hk1$; $hhl$, $h(h+1)l$. Crystals with their $b$ axis perpendicular to the plane of the needle were used for all photographs taken around (100) and for the first and second order photographs around (010). For the rest of the photographs crystals with their $b$ axis lying in the largest plane were used. From the Weissenberg and powder photographs the unit cell was found to be body-centered monoclinic with the axes: $a = 5.46_4$, $b = 4.88_7$ and $c = 11.8_1$ Å $\beta = 101.0°$ The lengths of the axes are almost the same as those given for the orthorhombic unit cell of $SbTaO_4$ (see above). As a close relation might be expected to exist between $SbTaO_4$ and $BiSbO_4$ the body-centered description will be used in the following. Except for the extinctions following from the body-centering, the only systematic extinction is that $h0l$ exists only for $h = 2n$ and $l = 2n$ which is characteristic of the space groups $C_{2h}^6 - I\,2/c$ and $C_s^4 - I\,c$.

Since only the C-centered descriptions are given in the International Tables (4) the point positions for the body-centered descriptions will be given here:

$C_s^4 - Ic$
$(000; \frac{1}{2}\frac{1}{2}\frac{1}{2}) +$
4: (a) $xyz$; $x\bar{y}(\frac{1}{2} + z)$

$C_{2h}^6 - I2/c$
$(000; \frac{1}{2}\frac{1}{2}\frac{1}{2}) +$
4: (a) $000$; $00\frac{1}{2}$ (b) $0\frac{1}{2}0$; $0\frac{1}{2}\frac{1}{2}$
(c) $\frac{1}{4}\frac{1}{4}\frac{1}{4}$; $\frac{1}{4}\frac{3}{4}\frac{3}{4}$
(d) $\frac{1}{4}\frac{1}{4}\frac{3}{4}$; $\frac{1}{4}\frac{3}{4}\frac{1}{4}$
(e) $0y\frac{1}{4}$; $0\bar{y}\frac{3}{4}$
8: (f) $xyz$; $\bar{x}\bar{y}\bar{z}$; $\bar{x}y(\frac{1}{2} - z)$; $x\bar{y}(\frac{1}{2} + z)$

The observed density for $BiSbO_4$ is 8.45 which agrees well with the assumption of 4 formula units/unit cell (calculated density 8.48).

## Positions of the metal atoms

All observed reflexions $hk(2n + 1)$ are weak or very weak. Of the reflexions $hk\,2n$, those of the type $h$, $k$, $n$ all odd or $h$, $k$, $n$ all even are strong, whereas the others are weak (see Table 2). This intensity distribution indicates that the positions of the heavy atoms are not far from the face-centered positions $000$; $\frac{1}{2}0\frac{1}{2}$; $0\frac{1}{2}\frac{1}{2}$; $\frac{1}{2}\frac{1}{2}0$ in a smaller unit cell with $a = a$, $b = b$ and $c = c/2$. This conclusion was supported by a study of the Patterson projections $P(xyp)$, $P(xpz)$ and $P(pyz)$ giving the following strong maxima: $\frac{1}{2}0$, $0\frac{1}{2}$, $\frac{1}{2}\frac{1}{2}$; $0\frac{1}{4}$, $\frac{1}{2}\frac{1}{4}$, $0\frac{1}{2}$, $\frac{1}{2}0$, $\frac{1}{2}\frac{1}{2}$; $0\frac{1}{4}$, $\frac{1}{2}\frac{1}{4}$, $0\frac{1}{2}$, $\frac{1}{2}0$, $\frac{1}{2}\frac{1}{2}$. With the above space groups we have the following approximate positions: 4 Sb or Bi ($Me_1$) in $000$, $\frac{1}{2}\frac{1}{2}\frac{1}{2}$, $00\frac{1}{2}$, $\frac{1}{2}\frac{1}{2}0$ and 4 Bi or Sb in $0\frac{1}{2}\frac{1}{4}$, $0\frac{1}{2}\frac{3}{4}$, $\frac{1}{2}0\frac{1}{4}$, $\frac{1}{2}0\frac{3}{4}$. With these positions, however, the intensities of the reflexions $hk(2n + 1)$ would only depend on the influence of the oxygen atoms, and this does not seem to be true since the strengths of the spots increase with increasing $k$ values, whereas the influence of the O atoms would be expected to decrease with increasing $\sin^2\theta$. It thus seems probable that a small deviation occurs from the above Me positions. To explain the $hk(2n + 1)$ reflexions small shifts in the $y$ parameters are necessary either for both Me positions or for one of them. Assuming $C_{2h}^6$, the only possible shift occurs for the $Me_2$ position (4($e$)). A Fourier synthesis was calculated for the $0kl$ spots with the arbitrary assumption of 4 Bi in 4($e$) and 4 Sb in 4($a$). The signs used corresponded to the assumption $\cos 2\pi ky < 0$ for $k = 1, 3, 5$. The value $0.51 - 0.53$ was obtained. Almost the same $y$ value was arrived at assuming 4 Bi in 4($a$) and 4 Sb in 4($e$). Thus for $C_{2h}^6$ two possibilities arise for the Me positions: 1. 4 Sb in 4($a$), 4 Bi in 4($e$) $y \approx 0.52$ and 2. 4 Bi in 4($a$), 4 Sb in 4($e$) $y \approx 0.52$. Though there is nothing in the photographs to indicate a lower symmetry than $C_{2h}^6$ small shifts in the above parameters seem to be possible without giving inacceptable intensity ratios. With $C_s^4$, 4 $Me_1$ can be arbitrarily assumed to occupy the position 4($a$) with $x = 0$ and $z = 0$. Intensity calculations (trial and error calculations on the $h0l$ and $0kl$ spots) gave the following approximate values: 4 $Me_1$ in 4($a$) $x = 0$, $y = 0.00 \pm 0.03$, $z = 0$; 4 $Me_2$ in 4($a$) $x = 0.00 \pm 0.03$, $y = 0.50 \pm 0.03$, $z = 0.25 \pm 0.01$.

## Positions of the O atoms

Since the $y$ values deviated only slightly from ideal values, the O atoms could be expected to exert some influence on the $hk(2n + 1)$ spots. With the

*Table 2*

Observed and calculated F values for the $0kl$, $1kl$ and $2kl$ Weissenberg photographs of $BiSbO_4$. $F_{obs}$ has been calculated from the formula $F^2_{obs} = I_{obs} \cdot \sin \Upsilon/(1 + \cos^2 2\theta)$. The $I_{obs}$ were estimated visually. For each of the rows $0kl$, $1kl$ and $2kl$ the estimated $F_{obs}$ values have been multiplied by a constant factor in order to attain as nearly as possible the same numerical values for $F_{obs}$ as for the corresponding $\lvert F_{calc}\rvert$. Under $F_{obs}{}^1$ are given the estimated $F_{obs}$ values obtained from the first order photographs taken around (001) and (110). $\lvert F_{Me}\rvert$ gives the $\lvert F_{calc}\rvert$ values when only the Me atoms are considered. $F_{calc} = 0.25\, \Sigma\, f \cos 2\pi\, (hx + ky + lz)$.

| $h\,k\,l$ | $F_{obs}$ | $F_{obs}{}^1$ | | $\lvert F_{Me}\rvert$ | $\lvert F_{calc}\rvert$ | $h\,k\,l$ | $F_{obs}$ | $F_{obs}{}^1$ | | $\lvert F_{Me}\rvert$ | $\lvert F_{calc}\rvert$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 0 2 | 24 | | | 31 | 23 | 0 6 2 | 0 | | | 14 | 14 |
| 0 0 4 | 74 | | | 114 | 105 | 0 6 4 | 71 | | | 57 | 57 |
| 0 0 6 | 31 | | | 27 | 23 | 1 1 0 | 10 | | | 28 | 22 |
| 0 0 8 | 88 | | | 91 | 86 | 1 1 2 | 92 | | | 113 | 108 |
| 0 0 10 | 26 | | | 22 | 31 | $1\,1\,\bar{2}$ | 83 | | | 116 | 116 |
| 0 0 12 | 63 | | | 72 | 72 | 1 1 4 | 26 | | | 27 | 26 |
| 0 0 14 | 0 | | | 19 | 14 | $1\,1\,\bar{4}$ | 31 | | | 28 | 38 |
| 0 1 1 | 11 | 12 | 9 | 6 | 10 | 1 1 6 | 103 | | | 94 | 101 |
| 0 1 3 | 20 | 19 | | 5 | 13 | $1\,1\,\bar{6}$ | 95 | | | 98 | 97 |
| 0 1 5 | 21 | 19 | | 5 | 12 | $1\,1\,\bar{8}$ | 27 | | | 25 | 22 |
| 0 1 7 | 0 | 6 | | 5 | 6 | $1\,1\,\overline{10}$ | 75 | | | 78 | 77 |
| 0 1 9 | 0 | 13 | | 4 | 9 | $1\,1\,\overline{12}$ | 17 | | | 20 | 20 |
| 0 1 11 | 0 | 0 | | 4 | 4 | $1\,1\,\overline{14}$ | 42 | | | 65 | 68 |
| 0 1 13 | 0 | 0 | | 3 | 6 | 1 2 1 | 9 | 6 | 0 | 10 | 6 |
| 0 2 0 | 82 | | | 107 | 96 | $1\,2\,\bar{1}$ | 17 | 8 | 0 | 10 | 13 |
| 0 2 2 | 21 | | | 27 | 22 | 1 2 3 | 13 | 11 | | 9 | 8 |
| 0 2 4 | 74 | | | 100 | 101 | $1\,2\,\bar{3}$ | 14 | 20 | | 10 | 16 |
| 0 2 6 | 25 | | | 25 | 22 | 1 2 5 | 20 | 18 | | 9 | 15 |
| 0 2 8 | 78 | | | 83 | 87 | $1\,2\,\bar{5}$ | 15 | 17 | | 9 | 13 |
| 0 2 10 | 0 | | | 20 | 16 | 1 2 7 | 0 | 17 | | 8 | 10 |
| 0 2 12 | 50 | | | 69 | 69 | $1\,2\,\bar{7}$ | 10 | 14 | | 8 | 9 |
| 0 3 1 | 30 | | 38 | 13 | 18 | $1\,2\,\bar{9}$ | 0 | 0 | | 8 | 0 |
| 0 3 3 | 0 | | | 13 | 8 | $1\,2\,\overline{11}$ | 0 | 0 | | 7 | 7 |
| 0 3 5 | 0 | | | 12 | 13 | $1\,2\,\overline{13}$ | 0 | 0 | | 6 | 10 |
| 0 3 7 | 0 | | | 11 | 9 | 1 3 0 | 27 | | | 24 | 28 |
| 0 3 9 | 0 | | | 10 | 11 | 1 3 2 | 99 | | | 88 | 90 |
| 0 3 11 | 0 | | | 10 | 13 | $1\,3\,\bar{2}$ | 95 | | | 89 | 91 |
| 0 3 13 | 0 | | | 9 | 7 | 1 3 4 | 21 | | | 23 | 27 |
| 0 4 0 | 115 | | | 80 | 76 | $1\,3\,\bar{4}$ | 9 | | | 23 | 13 |
| 0 4 2 | 18 | | | 20 | 17 | 1 3 6 | 56 | | | 78 | 70 |
| 0 4 4 | 112 | | | 76 | 80 | $1\,3\,\bar{6}$ | 56 | | | 80 | 82 |
| 0 4 6 | 30 | | | 17 | 22 | $1\,3\,\bar{8}$ | 17 | | | 20 | 23 |
| 0 4 8 | 104 | | | 70 | 68 | $1\,3\,\overline{10}$ | 53 | | | 69 | 69 |
| 0 4 10 | 0 | | | 17 | 14 | $1\,3\,\overline{12}$ | 17 | | | 18 | 19 |
| 0 5 1 | 18 | | 25 | 17 | 14 | 1 4 1 | 36 | | 28 | 15 | 22 |
| 0 5 3 | 32 | | | 17 | 18 | $1\,4\,\bar{1}$ | 18 | | 14 | 15 | 13 |
| 0 5 5 | 30 | | | 16 | 20 | 1 4 3 | 21 | | | 15 | 13 |
| 0 5 7 | 26 | | | 16 | 17 | $1\,4\,\bar{3}$ | 21 | | | 15 | 15 |
| 0 5 9 | 26 | | | 15 | 12 | 1 4 5 | 18 | | | 14 | 12 |
| 0 6 0 | 78 | | | 61 | 64 | | | | | | |

*Table 2* (cont).

| *h k l* | Fobs | Fobs[1] | \|FMe\| | \|Fcalc\| |
|---|---|---|---|---|
| 1 4 $\bar{5}$ | 21 | | 14 | 15 |
| 1 4 7 | 20 | | 14 | 15 |
| 1 4 $\bar{7}$ | 17 | | 14 | 11 |
| 1 4 $\bar{9}$ | 0 | | 13 | 18 |
| 1 4 $\overline{11}$ | 0 | | 12 | 11 |
| 1 5 0 | 30 | | 17 | 18 |
| 1 5 2 | 98 | | 67 | 69 |
| 1 5 $\bar{2}$ | 98 | | 68 | 65 |
| 1 5 4 | 18 | | 16 | 12 |
| 1 5 $\bar{4}$ | 33 | | 16 | 19 |
| 1 5 6 | 77 | | 63 | 65 |
| 1 5 $\bar{6}$ | 85 | | 65 | 62 |
| 1 5 $\bar{8}$ | 20 | | 16 | 17 |
| 1 6 1 | 30 | 16 | 18 | 18 |
| 1 6 $\bar{1}$ | 32 | 21 | 18 | 22 |
| 1 6 3 | 29 | | 18 | 15 |
| 1 6 $\bar{3}$ | 29 | | 18 | 22 |
| 2 0 2 | 42 | | 28 | 47 |
| 2 0 4 | 83 | | 98 | 100 |
| 2 0 $\bar{4}$ | 80 | | 104 | 100 |
| 2 0 6 | 18 | | 25 | 18 |
| 2 0 $\bar{6}$ | 25 | | 26 | 24 |
| 2 0 8 | 82 | | 80 | 80 |
| 2 0 $\bar{8}$ | 90 | | 87 | 99 |
| 2 0 10 | 0 | | 20 | 16 |
| 2 0 $\overline{10}$ | 20 | | 22 | 19 |
| 2 0 12 | 61 | | 67 | 72 |
| 2 0 $\overline{12}$ | 74 | | 71 | 67 |
| 2 0 $\overline{14}$ | 11 | | 19 | 19 |
| 2 1 1 | 14 | 14 16 | 5 | 11 |
| 2 1 $\bar{1}$ | 0 | 0 0 | 5 | 7 |
| 2 1 3 | 10 | — | 5 | 5 |
| 2 1 $\bar{3}$ | 18 | 20 | 5 | 12 |
| 2 1 5 | 17 | — | 4 | 11 |
| 2 1 $\bar{5}$ | 18 | 20 | 5 | 11 |
| 2 1 7 | 0 | — | 4 | 4 |
| 2 1 $\bar{7}$ | 11 | 0 | 4 | 7 |
| 2 1 9 | 0 | — | 4 | 2 |
| 2 1 $\bar{9}$ | 9 | 0 | 4 | 4 |
| 2 1 11 | 0 | — | 3 | 8 |
| 2 1 $\overline{11}$ | 0 | 0 | 4 | 6 |
| 2 1 13 | 0 | — | 3 | 2 |
| 2 1 $\overline{13}$ | 0 | 0 | 3 | 3 |
| 2 2 0 | 81 | | 98 | 104 |
| 2 2 2 | 12 | | 26 | 19 |
| 2 2 $\bar{2}$ | 19 | | 26 | 31 |
| 2 2 4 | 78 | | 88 | 89 |
| 2 2 $\bar{4}$ | 86 | | 94 | 94 |
| 2 2 6 | 26 | | 23 | 25 |
| 2 2 $\bar{6}$ | 23 | | 24 | 28 |
| 2 2 8 | 71 | | 74 | 71 |
| 2 2 $\bar{8}$ | 100 | | 80 | 75 |
| 2 2 10 | 18 | | 19 | 22 |
| 2 2 $\overline{10}$ | 20 | | 20 | 22 |
| 2 2 12 | 47 | | 64 | 62 |
| 2 2 $\overline{12}$ | 66 | | 68 | 69 |
| 2 2 $\overline{14}$ | 0 | | 18 | 17 |
| 2 3 1 | 11 | 0 0 | 12 | 13 |
| 2 3 $\bar{1}$ | 12 | 16 0 | 12 | 9 |
| 2 3 3 | 12 | 14 | 12 | 17 |
| 2 3 $\bar{3}$ | 17 | 27 | 12 | 13 |
| 2 3 5 | 0 | 0 | 11 | 8 |
| 2 3 $\bar{5}$ | 9 | 14 | 12 | 9 |
| 2 3 7 | 0 | 0 | 10 | 10 |
| 2 3 $\bar{7}$ | 0 | 23 | 11 | 14 |
| 2 3 9 | 0 | 6 | 9 | 10 |
| 2 3 $\bar{9}$ | 0 | 23 | 10 | 13 |
| 2 3 11 | 0 | 0 | 9 | 9 |
| 2 3 $\overline{11}$ | 0 | — | 10 | 7 |
| 2 3 $\overline{13}$ | 0 | — | 9 | 9 |
| 2 4 0 | 115 | | 74 | 71 |
| 2 4 2 | 24 | | 18 | 13 |
| 2 4 $\bar{2}$ | 33 | | 18 | 21 |
| 2 4 4 | 105 | | 71 | 67 |
| 2 4 $\bar{4}$ | 105 | | 72 | 75 |
| 2 4 6 | 22 | | 17 | 18 |
| 2 4 $\bar{6}$ | 21 | | 18 | 16 |
| 2 4 8 | 78 | | 65 | 67 |
| 2 4 $\bar{8}$ | 94 | | 67 | 63 |
| 2 4 10 | 0 | | 16 | 15 |
| 2 4 $\overline{10}$ | 12 | | 18 | 17 |
| 2 5 1 | 31 | 29 | 16 | 14 |
| 2 5 $\bar{1}$ | 39 | 36 | 16 | 18 |
| 2 5 3 | 28 | | 16 | 13 |
| 2 5 $\bar{3}$ | 42 | | 16 | 20 |
| 2 5 5 | 26 | | 16 | 16 |
| 2 5 $\bar{5}$ | 38 | | 16 | 16 |
| 2 5 7 | 19 | | 15 | 18 |
| 2 5 $\bar{7}$ | 22 | | 16 | 13 |

above Me positions the intensities of the *h k* (2 *n* + 1) spots would be constant ($C_{2h}^{6}$) or nearly constant ($C_{s}^{4}$) as long as *k* is constant. In fact a few discrepancies have been observed. Thus $013 > 011$, $015 > 017 < 019$, $141 > 14\bar{1}$, $211 > 21\bar{1}$ and $031 > 033$ (see Table 2). However, because of heavy absorption effects and since the influence of the O atoms on the majority of the spots is small, the determination of the positions of the O atoms by means of intensities only seemed likely to be a very difficult task. Possible positions were, therefore, sought for by space considerations, and intensity calculations (trial and error) were used to check the results. Since the normal configuration of O atoms around pentavalent Sb is an octahedron with distances $1.9 \leq \text{Sb–O} \leq 2.0$ Å, positions fulfilling these requirements were investigated. For these calculations the symmetry was first assumed to be $C_{2h}^6$ and the Me positions 4 (*a*) and 4 (*e*). With Sb in 4 (*e*) an octahedral arrangement around Sb seemed to be impossible for space reasons ($\text{O–O} < 2.5$ Å). Therefore, Sb was assumed to occupy the position 4 (*a*) and Bi 4 (*e*). With Sb surrounded by 6 O all of the O atoms of one 8 fold position must be in contact with 2 Sb (here called $O_1$ position), whereas for the remaining 8 O each O atom must be in contact with 1 Sb (here called $O_2$ position).

By systematically varying the $O_1$ positions, possible positions for the $O_2$ atoms were sought for. Care was taken that $\text{O–O} \geq 2.5$, $\text{Bi–O} \geq 2.2$, and $1.9 \leq \text{Sb–O} \leq 2.0$ Å. Varying the $O_1$ and $O_2$ positions led to configurations Bi–8 O for which 2 or 4 O were situated at short distances (2.2 – 2.3 Å) and the rest at distances $> 2.6$ Å. Thus no combination could be found for which Bi, like Sb in $SbTaO_4$, was closely surrounded by 6 O. Because the possible shifts in the Me parameters are small, the same result was obtained for the space group $C_s^4$. Other combinations, for instance Bi–5 O, possible only in the case of $C_s^4$ were not investigated since the structure finally arrived at with $C_{2h}^6$ seemed to be reasonable. Trial and error calculations made on $0kl$ and $hk1$ showed that though combinations giving one pair of $\text{Bi–O}_1$ distances equal to 2.6 Å could not be excluded by means of the intensities, better agreement was obtained for combinations giving $\text{Bi–O}_1$ distances = 2.9–3.0 Å.

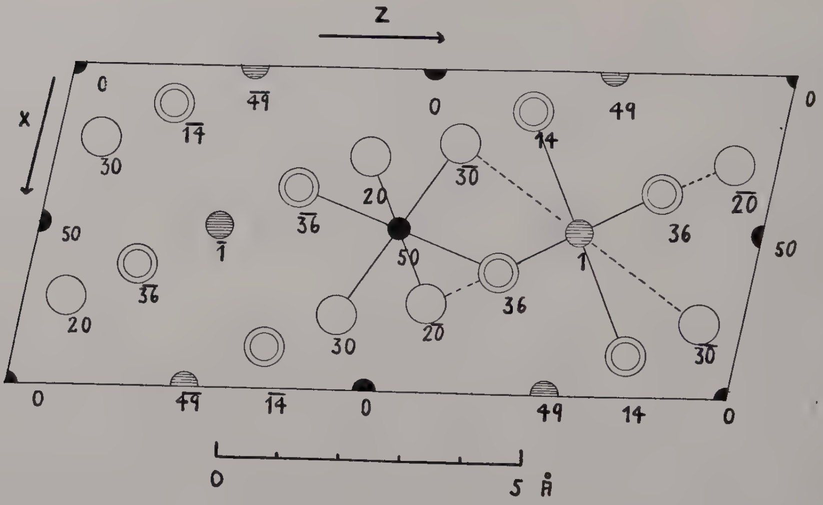
The following parameters were finally arrived at: $y_{\text{Bi}} = 0.51_2$ O atoms: $x_1 = 0.23$ $y_1 = 0.30$ $z_1 = 0.06$, $x_2 = 0.12$ $y_2 = -0.14$ $z_2 = 0.15$. For the $0kl$, $1kl$ and $2kl$ spots the quotient: $\Sigma \big|\, |F_{\text{obs}}| - |F_{\text{calc}}| \,\big| / \Sigma |F_{\text{obs}}|$ has the value 0.33 when the Me atoms only are considered and 0.31 when the O atoms are also considered. Thus a slight improvement results from calculations which also involve the O atoms. For the above calculations the $F_{\text{obs}}$ too weak to be observed have been assumed to have the value zero. The high value of the quotient is probably partly due to absorption effects for which no corrections have been made.

The following structure is proposed:

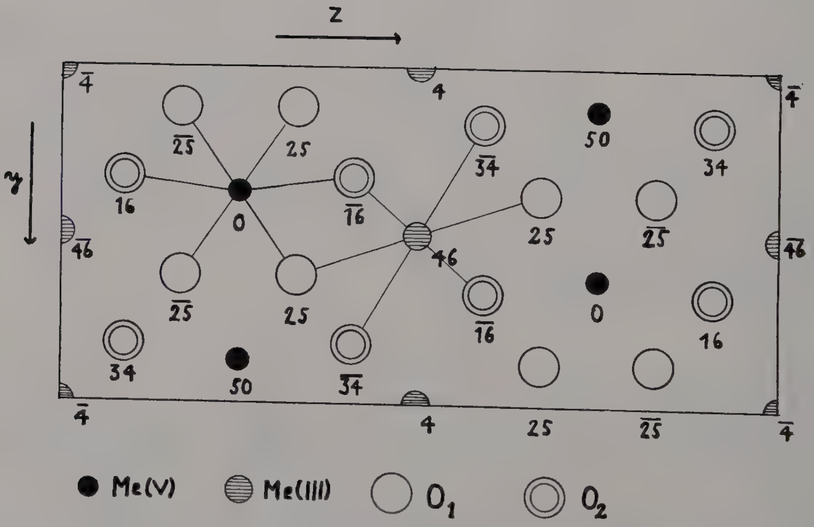
$C_{2h} - I\,2/c$
$(0\,0\,0;\ \tfrac{1}{2}\,\tfrac{1}{2}\,\tfrac{1}{2})\ +$
4 Sb in 4 (a) $0\,0\,0;\ 0\,0\,\tfrac{1}{2}$
4 Bi in 4 (e) $0\,y\,\tfrac{1}{4};\ 0\,\bar{y}\,\tfrac{3}{4}$ $y = 0.51_2$
8 $O_1$ in 8 (f) $x\,y\,z;\ \bar{x}\,\bar{y}\,\bar{z};\ \bar{x}\,y\,(\tfrac{1}{2} + z);\ x\,\bar{y}\,(\tfrac{1}{2} - z)$ $x_1 = 0.23$ $y_1 = 0.30$ $z_1 = 0.06$
8 $O_2$ in 8 (f) $x_2 = 0.12$ $y_2 = -0.14$ $z_2 = 0.15$

The distances (Å) and coordination will be:

| | | |
|---|---|---|
| $\text{Sb–4 O}_1$ | $2 \cdot 2.0_1$, | $2 \cdot 1.9_7$ |
| $\text{Sb–2 O}_2$ | $1.9_0$ | |
| $\text{Bi–4 O}_1$ | $2 \cdot 2.8_7$, | $2 \cdot 2.9_6$ |
| $\text{Bi–4 O}_2$ | $2 \cdot 2.2_5$, | $2 \cdot 2.2_7$ |


Z
X
0
30
$\overline{14}$
$\overline{49}$
0
49
14
$\overline{30}$
20
50
$\overline{36}$
$\overline{1}$
$\overline{20}$
36
1
50
$\overline{36}$
20
30
$\overline{20}$
36
$\overline{30}$
0
$\overline{49}$
$\overline{14}$
0
49
14
0
0
5 Å

1 a


Z
Y
$\overline{4}$
4
$\overline{4}$
$\overline{25}$
25
16
0
$\overline{16}$
$\overline{34}$
50
34
46
$\overline{46}$
25
$\overline{25}$
$\overline{46}$
$\overline{25}$
25
$\overline{16}$
0
16
34
50
$\overline{34}$
$\overline{4}$
4
25
$\overline{25}$
$\overline{4}$
Me(V)
Me(III)
O$_1$
O$_2$

1 b

Fig. 1 a and 1 b. 1 a. Projection of the structure of BiSbO$_4$ on the $xz$ plane. 1 b. Projection of the structure of SbTaO$_4$ on the $yz$ plane. The heights in the direction perpendicular to the plane of the paper are given in hundredths of the corresponding axes.

| | | |
|---|---|---|
| $O_1 - 4\,O_1$ | $2 \cdot 2.7_8$, | $2.8_5$, $(3.2_8)$ |
| $O_2 - 4\,O_2$ | $2 \cdot 2.9_4$, | $2.7_1$, $2.9_1$ |
| $O_1 - 4\,O_2$ | $2.5_2$, $2.7_5$, $2.7_7$, $2.9_3$ | |

A projection of the structure on the *xz* plane is given in Figure 1 a. As may be seen Sb is surrounded by a somewhat deformed octahedron of O atoms with the edges $2.5_2$, $2.7_5$, $2.7_7$, $2.7_8$, $2.8_5$ and $2.9_3$ Å. Every Bi is surrounded by 8 O, four of which ($O_2$) lie on one side (in the *y* direction) at short distances (2.2 – 2.3 Å) and four on the other side at larger distances (2.9 – 3.0 Å). The $O_2$ atoms form chains of puckered tetragons parallel to the *x* axis. The Bi atoms are situated alternatingly above and below the tetragons (see Figure 1 a). In $SbTaO_4$ (see Figure 1 b) Sb is surrounded by 6 O at short distances. Four of the O atoms ($O_2$) surrounding one Sb atom lie on one side of the Sb atom (in the *x* direction) and the Sb and O atoms form chains along the *y* axis of the same type as those mentioned above. Me–O or Me–OH chains of the same kind have been met with in other structures, for instance Th–OH in $Th(OH)_2CrO_4 \cdot H_2O$ (5) and Pb–O in $Ag_2PbO_2$ (6).

The author wishes to thank Professor L. G. Sillén for valuable discussions on this work.

Stockholms Högskola, Institute of Inorganic and Physical Chemistry, January 1951.

REFERENCES. 1. **Dihlström, K.,** Z. anorg. Ch. *239* (1938), 57. — 2. Dana's System of Mineralogy, New York 1944, seventh edition, vol. 1 p. 770. — 3. **Brauer, G.** Z. anorg Ch. *248*, (1941), 1. — 4. Internationale Tabellen zur Bestimmung von Kristallstrukturen, Berlin 1935. — 5. **Lundgren, G.** and **Sillén, L. G.,** Arkiv Kemi *1*, (1949), 277. — 6. **Byström, A.** and **Evers, L.,** Acta Chem. Scand. *4* (1950), 613.

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# The configuration of optically active α-methyl-α-ethylsuccinic acid

## By JERKER PORATH

With 4 figures in the text

Optically active α-methyl-α-ethylsuccinic acid was synthesized by STÄLLBERG-STENHAGEN (1) as an intermediate product in the synthesis of optically active α-methyl-α-ethylsubstituted fatty acids. The object of the present investigation is to relate this acid to the FISCHER conventional system.

The tendency of optically active monoalkylsuccinic acids to form either quasiracemic compounds or simple eutectic mixtures with one another can be utilized for establishment of sterical relationships. The method can be extended to dialkylsuccinic acids. In these cases, however, caution must be taken. It is not convenient, for example, to use methylsuccinic acid for comparison with α-methyl-α-ethylsuccinic acid as it is not quite clear if the methyl group in this acid corresponds to the methyl or to the ethyl group of the α-methyl-α-ethylsuccinic acid. It seems, however, to be a sound assumption that the ethyl group of ethylsuccinic acid corresponds sterically to that of α-methyl-α-ethylsuccinic acid.

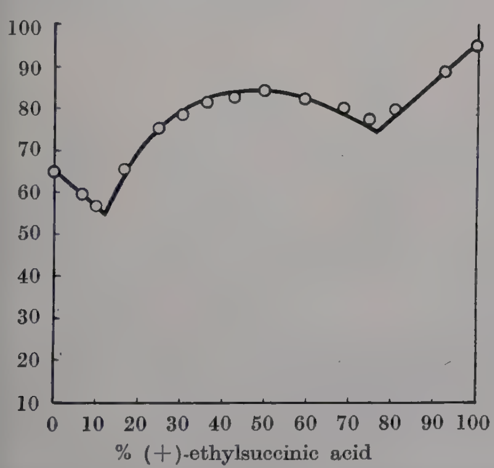


Fig. 1. (−)-α-methyl-α-ethylsuccinic acid and (+)-ethylsuccinic acid.

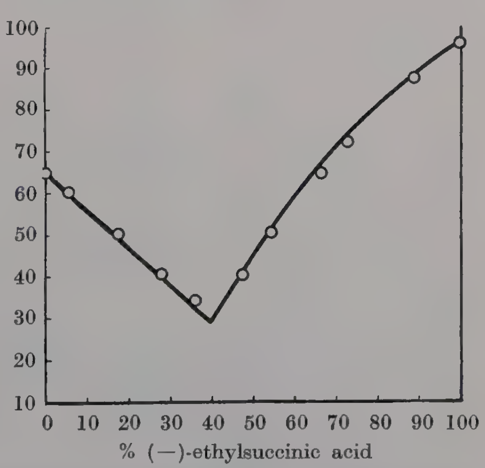


Fig. 2. (−)-α-methyl-α-ethylsuccinic acid and (−)-ethylsuccinic acid.

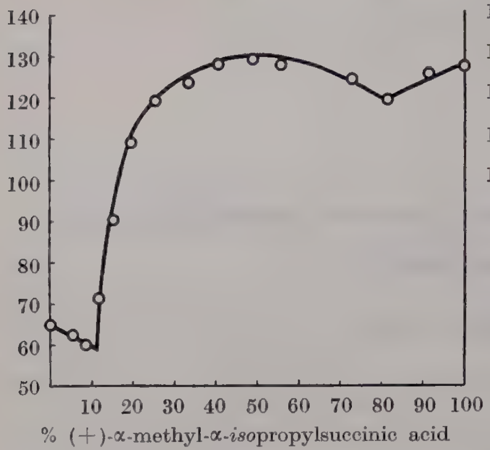


Fig. 3. (+)-α-methyl-α-*iso*propylsuccinic acid and (—)-α-methyl-α-ethylsuccinic acid.

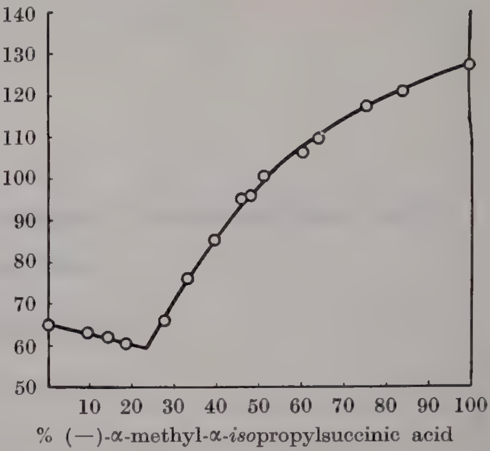
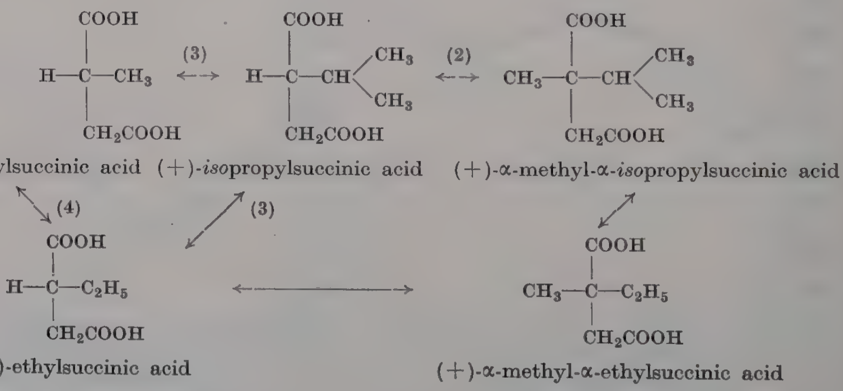


Fig. 4. (—)-α-methyl-α-*iso*propylsuccinic acid and (—)-α-methyl-α-ethylsuccinic acid.

The melting point diagram of (+)-ethylsuccinic acid with (—)-α-methyl-α-ethylsuccinic acid (fig. 1) shows the formation of a molecular compound, whereas (—)-ethylsuccinic acid with (—)-α-methyl-α-ethylsuccinic acid form only a simple eutectic mixture (fig. 2). Thus the antipodes with the same direction of rotation have the same configuration.

Two years ago the configuration of optically active α-methyl-α-isopropylsuccinic acid was determined by the melting point diagram method (2). It is of some interest to compare this acid with α-methyl-α-ethylsuccinic acid. The diagrams 3 and 4 show the formation of a molecular compound between (+)-α-methyl-α-isopropylsuccinic acid and (—)-α-methyl-α-ethylsuccinic acid and a simple eutectic mixture, respectively.

Some substances related to α-methyl-α-ethylsuccinic acid can now be compiled in the following scheme, the formulae being written according to the FISCHER convention:


COOH
H—C—CH₃
CH₂COOH
(3)
COOH
H—C—CH(CH₃)(CH₃)
CH₂COOH
(2)
COOH
CH₃—C—CH(CH₃)(CH₃)
CH₂COOH
(+)-methylsuccinic acid
(+)-*iso*propylsuccinic acid
(+)-α-methyl-α-*iso*propylsuccinic acid
(4)
(3)
COOH
H—C—C₂H₅
CH₂COOH
COOH
CH₃—C—C₂H₅
CH₂COOH
(+)-ethylsuccinic acid
(+)-α-methyl-α-ethylsuccinic acid

(The sign ←→ indicates that the connection was established by means of the melting point diagram method.)

## Experimental

The melting point diagrams were obtained according to RHEINBOLDT (5) and by using FRIEDRICHS' melting point apparatus (6).

*Table 1*

(+)-ethylsuccinic acid and (—)-α-methyl-α-ethylsuccinic acid.

| % (+)-ethylsuccinic acid | Melting point |
|---|---|
| 0 | 65°0 |
| 6.8 | 59.5 |
| 9.8 | 57.0 |
| 16.5 | 65.5 |
| 24.9 | 75.5 |
| 30.3 | 78.5 |
| 35.7 | 81.5 |
| 42.3 | 83.0 |
| 49.4 | 84.5 |
| 59.1 | 82.5 |
| 68.2 | 80.5 |
| 74.8 | 78.0 |
| 80.8 | 80.0 |
| 92.3 | 89.5 |
| 100.0 | 96.5 |

*Table 2*

(—)-ethylsuccinic acid and (—)-α-methyl-α-ethylsuccinic acid.

| % (—)-ethylsuccinic acid | Melting point |
|---|---|
| 0 | 65°0 |
| 5.4 | 60.5 |
| 17.2 | 50.5 |
| 27.9 | 41.0 |
| 35.8 | 34.5 |
| 47.1 | 40.5 |
| 54.0 | 50.5 |
| 66.2 | 65.0 |
| 72.5 | 72.5 |
| 88.8 | 88.0 |
| 100.0 | 96.5 |

*Table 3*

(—)-α-methyl-α-ethylsuccinic acid and (+)-α-methyl-α-*iso*propylsuccinic acid.

| % (+)-α-methyl-α-*iso*-propylsuccinic acid | Melting point |
|---|---|
| 0 | 65°0 |
| 5.3 | 62.5 |
| 8.3 | 60.0 |

Table 3 (cont.)

| % (+)-α-methyl-α-*iso*-propylsuccinic acid | Melting point |
|---|---|
| 11.9 | 71°5 |
| 15.1 | 90.5 |
| 19.8 | 109.0 |
| 25.5 | 119.0 |
| 33.5 | 123.5 |
| 40.7 | 127.5 |
| 49.2 | 129.0 |
| 55.9 | 127.5 |
| 73.3 | 124.0 |
| 81.5 | 119.0 |
| 91.4 | 125.0 |
| 100.0 | 127.0 |

*Table 4*

(—)-α-methyl-α-ethylsuccinic acid and (—)-α-methyl-α-*iso*propylsuccinic acid.

| % (—)-α-methyl-α-*iso*-propylsuccinic acid | Melting point |
|---|---|
| 0 | 65°0 |
| 9.1 | 63.0 |
| 14.2 | 62.0 |
| 18.3 | 60.5 |
| 27.5 | 66.0 |
| 33.2 | 76.0 |
| 39.1 | 85.0 |
| 46.0 | 95.0 |
| 47.8 | 95.5 |
| 51.2 | 100.5 |
| 60.4 | 105.5 |
| 63.9 | 109.5 |
| 75.3 | 117.0 |
| 83.9 | 120.5 |
| 100.0 | 127.0 |

Professor ARNE FREDGA and Doctor STINA STÄLLBERG-STENHAGEN kindly put at my disposal active ethylsuccinic acid and α-methyl-α-ethylsuccinic acid, respectively. I take this opportunity of expressing my sincere gratitude for this and for their interest.

Organic Department, Chemical Institute, University of Uppsala, January 1951.

REFERENCES: 1. **S. Ställberg-Stenhagen,** In print. — 2. **J. Porath,** Arkiv Kemi, *Bd 1,* n:o 45 (1949). — 3. **A. Fredga** and **E. Leskinen,** Arkiv Kemi, Min. Geol. *Bd 19,* n:o 1 (1944). — 4. **A. Fredga,** "The Svedberg 1884 $^{30}/_{8}$ 1944", 261–273 (Uppsala 1944). — 5. **H. Rheinboldt,** J. Prakt. Chem. [2], *111,* 242 (1925). — 6. **F. Friedrichs,** Z. angew. Chem. *34,* 61 (1921).

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# The isotope distribution in the rapidly metabolising fatty acid fraction of the liver

By G. A. SWAN

Recent work carried out by HEVESY, RUYSSEN and BEECKMANNS (1, 2), in which 1—$^{14}$C-acetate was injected intraperitoneally into mice, has shown that strong activities in the cholesterol and (more especially) the fatty acids of the liver are demonstrable after as short a time as 15 minutes and much of the acitivity of the fatty acids observed in the early stage of the experiment is no longer present after the lapse of 1 hour. The half-lifetime of the average fatty acid of the liver of the rat is known to be about 1—2 days, which presumably does not much differ from the corresponding value for the mouse. It was therefore suggested that the liver of the mouse must contain a rapidly metabolising fatty acid fraction. At first, it was thought possible that the very rapid appearance of activity in the fatty acids might be due simply to an interchange of carboxyl groups between the endogenous fatty acids and the exogenous (labelled) acetate. This is not, however, the case, as is shown by the results of the experiments described in this note, on the saturated fatty acids formed by decomposition of the lead salts isolated by Dr. BEECKMANNS and Miss ELLIOTT (2).

For comparison of the isotope concentration of the carboxyl group with that of the total fatty acid molecule, previous workers, e.g., RITTENBERG and BLOCH (3), ANKER (4), have decarboxylated fatty acids by heating to a temperature of 300—370° with iron filings, following the method of EASTERFIELD and TAYLOR (5). DAUBEN, REID, YANKWICH and CALVIN (6) have shown that the decarboxylation of phenylacetic acid with copper chromite and quinoline at 230° leads to some interchange of the carboxyl and $\alpha$-carbon atoms. While, of course, these two decarboxylations are not strictly analogous, nevertheless it appears that a milder method is desirable. DOERING, TAYLOR and SCHOENEWALDT (7) have used the Schmidt reaction for the decarboxylation of labelled benzoic acid. This method has now been applied to the fatty acids. Unfortunately, however, it was found that the evolved carbon dioxide is contaminated with sulphur dioxide, the latter presumably arising through reduction of the sulphuric acid by hydrazoic acid, which is known to be formed by the action of concentrated sulphuric acid on hydrazoic acid. Thus, when the evolved gases are passed into barium hydroxide solution, the precipitated barium carbonate is contaminated with barium sulphite. (In some experiments, the weight of barium sulphite was as great as one-third that of barium carbonate.) However, after the radioactivity measurements had been carried out, the amount of barium sulphite in the sample was determined by oxidation to barium sulphate, which was estimated gravimetrically; and the activity values were then corrected. The amines produced in the reaction were also isolated. Schmidt (8) showed that the action of hydrazoic acid and sulphuric acid on benzene at 60° gave aniline; so there is risk of contamination of the amines by aniline, when benzene is used as solvent.

The results obtained are shown in the accompanying table. In the long-term feeding experiments on rats by Rittenberg and Bloch (3), ratio A was found to be slightly less than 2. Here, in the very short-term experiments, A has a value of approximately 3, falling to 1.8 in the longer (100 minute) experiments. This could be explained by reaction between endogenous (unlabelled) fatty acids and exogenous (labelled) acetate at the beginning of the experiment resulting in higher labelling of the carboxyl groups than of the rest of the molecules of the fatty acids. Soon, however, the whole molecules would become uniformly labelled at alternate carbon atoms. Ratio B also confirms the labelling of the molecule as a whole.

| Duration of experiment (minutes) | 15 | 30 | 45 | 70 | 100 |
|---|---|---|---|---|---|
| Activity of $BaCO_3$ from combustion of acids (c./min.) | 53.6 | 25.3 | 49.7 | 40.0 | 35.0 |
| Activity of $BaCO_3$ from combustion of amines | 44.0 | 23.9 | 42.4 | 31.6 | 28.3 |
| $\frac{\text{Isotope concentration of carboxyl group}}{\text{Isotope concentration of acid}} = A$ |  | 2.95 | 2.3 |  | 1.8 |
| $\frac{\text{Isotope concentration of amine}}{\text{Isotope concentration of acid}} = B$ | 0.82 | 0.94 | 0.85 | 0.79 | 0.81 |

Thus, the work carried out with the samples obtained from Beeckmanns and Elliott clearly indicates that in the rapid formation of labelled fatty acid molecules the whole molecule is involved, although in addition to this main effect, another is superimposed in which carboxyl carbon is involved. The last mentioned effect is observable in experiments of very short duration only.

## Experimental

A solution of the fatty acid (25—30 mg) in benzene (1 ml) was treated with concentrated sulphuric acid (0.2 ml) in a reaction-vessel fitted with a mercury-seal stirrer, nitrogen inlet and outlet tubes, and a short side-arm, attached via a short piece of rubber tubing to a small tube containing sodium azide (80 mg). A slow stream of pure, dry nitrogen was passed first through a vessel containing benzene, then through the reaction vessel, both vessels being in the same water-bath at 43—46°. When the air had been displaced from the apparatus, 1.5 ml of a solution containing 1.5 % barium hydroxide and 2 % of barium chloride was introduced into a small absorption vessel connected to the nitrogen-outlet of the reaction vessel. The contents of the reaction vessel were stirred vigorously, while the sodium azide was gradually added from the side tube during 45 minutes. Stirring, with passage of nitrogen, was continued for a further 45 minutes. The barium carbonate (contaminated with barium sulphite) was collected by centrifugation under ether, washed similarly once with 2 % barium chloride solution and twice with carbon dioxide-free distilled water, and dried for 4 hours at 130°. After the activity measurements had been carried out, the sample was weighed accurately into a small centrifuge tube. Distilled water (0.5 ml) was added, and the solid centrifuged to the bottom of the tube. Dilute hydrochloric acid (3 drops) was added and the tube immediately closed by a stopper. When all the precipitate had dissolved, the stopper was removed, excess of bromine water was added, and the stopper was again inserted immediately. After 10 minutes, the stopper was removed and the solution heated to boiling point. The precipitated barium sulphate was then filtered through a previously ignited and weighed micro Gooch crucible (platinum). After being washed, the precipitate was ignited to constant weight.

The amines formed in the Schmidt reaction were isolated from the contents of the reaction vessel by dilution with water, rendering alkaline by sodium hydroxide, and extraction with benzene. The benzene extract was cleared by centrifugation, washed with 40 % sodium hydroxide solution and dried with potassium carbonate. Filtration and evaporation yielded a gum which solidified at room temperature.

The samples were combusted in the presence of platinum and oxygen at 850°, and the derived barium carbonate treated as above.

The activity measurements were carried out with a Geiger end-window counter, using "infinitely thick" layers of barium carbonate (6 mg) in dishes, 4.5 mm in diameter. The values given are corrected for background.

## SUMMARY

The liver fatty acids of mice, isolated short times (15—100 minutes) after the intraperitoneal injection of 1—$^{14}$C-acetate, were subjected to the Schmidt reaction. The isotope concentration of the evolved carbon dioxide, the original acids and the amines formed were compared. The results suggest that the $^{14}$C is uniformly distributed at alternate carbon atoms throughout the whole fatty acid molecule, except in the acids isolated from the shortest term experiments; in these latter acids there appears to be some excess of $^{14}$C in the carboxyl group.

Thanks are offered to Professor G. Hevesy for his interest in this work.

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REFERENCES. 1. **Hevesy, Ruyssen** and **Beeckmanns,** Exper., in the press. — 2. **Beeckmanns** and **Elliott,** Nature, 1951, *167,* 200. — 3. **Rittenberg** and **Bloch,** J. Biol. Chem., 1944, *154,* 311. — 4. **Anker,** J. Biol. Chem., 1948, *176,* 1337. — 5. **Easterfield** and **Taylor,** J. Chem. Soc., 1911, *99,* 2298. — 6. **Dauben, Reid, Yankwich** and **Calvin,** J. Amer. Chem. Soc., 1950, *72,* 121. — 7. **Doering, Taylor** and **Schoenewaldt,** J. Amer. Chem. Soc., 1948, *70,* 455. — 8. **Schmidt,** Ber., 1924, *57,* 704.

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# On cyclohexyl compounds with quadri- and sexi-valent sulphur

By Bengt Weibull

## I. Introduction and survey

In a previous paper (1) the author described the preparation and properties of cyclohexanethiol and some cyclohexyl sulphides. The aim of this work is to describe the preparation and properties of cyclohexyl compounds obtained from the foregoing ones by reactions involving the sulphur atom, i.e. sulphonium compounds, sulphoxides, sulphones, and derivatives related to them or prepared from them by simple reactions.

**Cyclohexylsulphonium compounds.** These compounds are of special interest in that some of them may be degraded to sulphur-free derivatives of cyclohexane. They were prepared by alkylation of the sulphides with dimethyl sulphate, methyl iodide, or halogenoacetic acid.

Using methyl iodide without any solvent, neutral, crystalline sulphonium iodides were formed with cyclohexyl methyl sulphide and dicyclohexyl sulphide, but with cyclohexyl benzyl sulphide the reaction product did not crystallise, and with cyclohexyl phenyl sulphide no reaction was observed. The iodides were rather stable at 100° in aqueous solution of acid, neutral, or alkaline reaction. When refluxed with an equimolecular amount of ethanolic potassium hydroxide they were decomposed. Cyclohexyldimethylsulphonium hydroxide thus yielded mainly cyclohexyl methyl sulphide (and probably methanol), but also some dimethyl sulphide (and probably cyclohexene). Dicyclohexylmethylsulphonium hydroxide as a main product gave dicyclohexyl sulphide (and probably methanol) and small amounts of cyclohexyl methyl sulphide and cyclohexene. Ingold et al. (see 2) made extensive investigations concerning the degradation of sulphonium compounds. They did not use cyclohexyl compounds and their conditions of reaction were not strictly comparable, but as they in several cases obtained olefins in high yields, it seemed rather unexpected, that cyclohexene was only a minor product of the reactions mentioned.

Cyclohexylsulphonium compounds containing carboxyl groups were prepared either by methylation of cyclohexylthio-carboxylic acids or by reaction of cyclohexyl sulphides with halogenoacetic acid. The following compounds were thus prepared: cyclohexylmethylcarboxymethylsulphonium bromide, cyclohexylmethyl-2-carboxyethylsulphonium iodide, cyclohexylcarboxymethylthetin, cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide and chloride, and the corresponding thetin, cyclohexyl-2-carboxyethylthetin.

Cyclohexylmethylcarboxymethylsulphonium bromide was prepared from cyclohexyl methyl sulphide and bromoacetic acid. Using methyl iodide and cyclohexylthioacetic acid without any solvent, only resinous products resulted, but with this acid and dimethyl sulphate in aqueous medium a sulphonium salt was obtained, isolated as the complex of the chloride with mercuric chloride. Several unsuccessful attempts were made to prepare cyclohexylmethylthetin from the corresponding sulphonium salts. These were rather stable in aqueous solution of acid, neutral, or alkaline reaction, and thus resemble the corresponding methyl-, ethyl-, and n-propyl-compounds investigated by HOLMBERG (3). When refluxed with two moles of ethanolic potassium hydroxide the bromide was decomposed yielding cyclohexyl methyl sulphide, traces of dimethyl sulphide, salts of cyclohexylthio- and methylthio-acetic acid, cyclohexene, carbonate and (probably) cyclohexyldimethylsulphonium ions. The degradation may be accounted for by the scheme:

1 a. $C_6H_{11}S^+(CH_3)CH_2COO^- + OH^- \rightarrow C_6H_{11}S(CH_3)_2^+ + CO_3^{--}$

1 b. $C_6H_{11}S^+(CH_3)CH_2COO^- + OH^- \rightarrow C_6H_{11} \cdot S \cdot CH_2COO^- + CH_3OH$

1 c. $C_6H_{11}S^+(CH_3)CH_2COO^- + OH^- \rightarrow CH_3 \cdot S \cdot CH_2COO^- + C_6H_{10} + H_2O.$

The cyclohexyldimethylsulphonium ions formed according to reaction 1 a. were then partly decomposed as described above.

Cyclohexylcarboxymethylthetin was easily prepared in neutral aqueous solution from the sodium salts of cyclohexylthioacetic acid and bromoacetic or chloroacetic acid. It was rather stable when heated in alkaline, aqueous solution. Comparable investigations have only been carried out with 2-phenylethylcarboxymethylthetin, which yielded styrene (4). In hot water and hot, dilute hydrochloric acid it was decomposed with formation of cyclohexene, cyclohexanol (traces), and carboxymethylthioacetic acid:

2 a. $C_6H_{11}S^+(CH_2COOH)_2 \rightarrow C_6H_{10} + S(CH_2COOH)_2 + H^+$

2 b. $C_6H_{11}S^+(CH_2COOH)_2 + H_2O \rightarrow C_6H_{11}OH + S(CH_2COOH)_2 + H^+.$

When heated above the melting point, the compound was rapidly decomposed affording a variety of neutral compounds, some of which were not identified. The products identified included cyclohexyl methyl sulphide (main product), dimethyl sulphide, methyl esters of cyclohexylthio- and carboxymethylthioacetic acid, cyclohexene, and carbon dioxide. When the fraction containing methyl cyclohexylthioacetate was saponified, there was also formed some cyclohexanethiol and formaldehyde. The following scheme is an attempt to account for the main products:

3 a. $C_6H_{11}S^+(CH_2COO^-)CH_2COOH \rightarrow C_6H_{10} + S(CH_2COOH)_2$

3 b. $C_6H_{11}S^+(CH_2COO^-)CH_2COOH \rightarrow C_6H_{11} \cdot S \cdot CH_2COOCH_3 + CO_2$

3 c. $C_6H_{11}S^+(CH_2COO^-)CH_2COOH \rightarrow C_6H_{10} + (CH_3)_2S + 2\,CO_2$

3 d. $C_6H_{11}S^+(CH_2COO^-)CH_2COOH \rightarrow C_6H_{11}SCH_3 + CH_2 + 2\,CO_2.$

The methylene radical postulated in 3 d. methylates the acid formed in 3 a. The thermal decomposition of a carboxymethylthetin seems not to have been investigated before. With dimethylthetin LETTS (5) obtained trimethylsulphonium carbonate and carbon dioxide, and with biscarboxymethylthetin DELISLE (6) obtained dimethyl sulphide, the methyl esters of methylthio- and carboxymethylthio-acetic acid, and carbon dioxide.

Cyclohexylmethyl-2-carboxyethylsulphonium iodide was prepared by methylation of 2-cyclohexylthiopropionic acid with methyl iodide. Attempts to convert it to the corresponding thetin were unsuccessful. The sulphonium salt was rather stable in hot, acid or neutral, aqueous solution. In alkaline solution it was rapidly decomposed in analogy with the corresponding methyl- and benzyl-compounds, described by CARRARA (7) and HOLMBERG (8), yielding cyclohexyl methyl sulphide and acrylate:

4. $C_6H_{11}S^+(CH_3)CH_2CH_2COO^- + OH^- \rightarrow C_6H_{11}SCH_3 + CH_2 = CHCOO^- + H_2O$.

Cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide (chloride) was formed in reaction between 2-cyclohexylthiopropionic acid and bromoacetic (chloroacetic) acid in concentrated aqueous solution. The corresponding thetin was obtained from it by means of silver oxide or ethanolic potassium hydroxide. The thetin could not be prepared by the method used for the preparation of cyclohexylcarboxymethylthetin. In alkaline solution it was decomposed in analogy with formula 4., salts of cyclohexylthioacetic and acrylic acid being formed (compare also 9). In hot aqueous solution the bromide gave a variety of products including cyclohexene, cyclohexanol (traces), 2-carboxymethylthiopropionic acid, cyclohexylthioacetic acid, and acrylic acid. These products may be accounted for by schemes, analogous to 2. and 4. In hot, aqueous solution with one mole of alkali (corresponding to a solution of the thetin) the same products were obtained, but also some neutral material that possibly consisted of methyl cyclohexylthioacetate, and carbon dioxide.

Attempts were also made to prepare sulphonium compounds from bromoacetic acid and other neutral cyclohexyl sulphides. With dicyclohexyl sulphide the reaction product did not crystallise, and with cyclohexyl benzyl and cyclohexyl phenyl sulphide no reaction was observed. Attempts were also made to prepare sulphonium compounds derived from cyclohexylthiosuccinic acid, but they were unsuccessful.

**Cyclohexyl sulphoxides.** These were prepared by oxidation of the corresponding sulphides, in acetone, with hydrogen peroxide. Cyclohexyl methyl sulphoxide at room temperature was a hygroscopic oil, the other ones crystalline compounds. Cyclohexylsulphinylacetic acid when heated alone, with water, or dilute hydrochloric acid was decomposed according to the scheme (for a survey of this reaction see (10)):

5. $C_6H_{11} \cdot SO \cdot CH_2COOH \rightarrow C_6H_{11}SH + OHC \cdot COOH$.

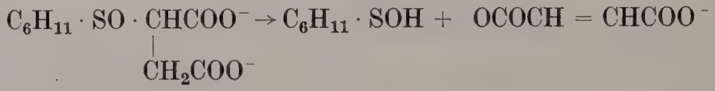
In a secondary reaction there was formed biscyclohexylthioacetic acid.

2-Cyclohexylsulphinylpropionic acid in warm, alkaline solution was decomposed according to the scheme (compare HOLMBERG (9) and (11)):

6. $C_6H_{11} \cdot SO \cdot CH_2CH_2COO^- + OH^- \rightarrow C_6H_{11} \cdot SO^- + CH_2 = CHCOO^- + H_2O$.

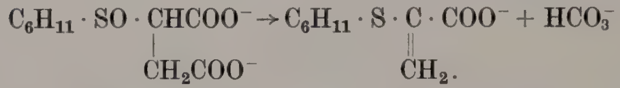
In a secondary reaction the sulphenate was disproportionated to cyclohexanesulphinate and dicyclohexyl disulphide. (For a survey of the properties of sulphenic acids see (12).) In warm, acid solution the decomposition proceeded more slowly, but the corresponding products were formed.

Cyclohexylsulphinylsuccinic acid (optically inactive throughout the work) finally under similar conditions yielded a variety of compounds. In warm, alkaline solution the decomposition proceeded in analogy with formula 6., yielding cyclohexanesulphinate, dicyclohexyl disulphide, and fumarate. In warm, neutral solution a slow reaction led to the formation of an inseparable mixture of dicyclohexyl disulphide and trisulphide (or sulphur), fumarate, cyclohexanone, acid carbonate, and 1-cyclohexylthioacrylate. The last compound is further investigated in another work (13). Cyclohexanesulphinic acid was sought for in vain. The mechanisms for the formation of the compounds are obscure, but the reactions may easily be formulated:

7 a.


$$C_6H_{11} \cdot SO \cdot \underset{\displaystyle CH_2COO^-}{\underset{|}{CHCOO^-}} \rightarrow C_6H_{11} \cdot SOH + \ OCOCH = CHCOO^-$$

7 b. $3\,C_6H_{11} \cdot SOH \rightarrow C_6H_{11} \cdot SS \cdot C_6H_{11} + S + C_6H_{10}O + 2\,H_2O$

7 c.


$$C_6H_{11} \cdot SO \cdot \underset{\displaystyle CH_2COO^-}{\underset{|}{CHCOO^-}} \rightarrow C_6H_{11} \cdot S \cdot \underset{\displaystyle CH_2.}{\underset{\|}{C}} \cdot COO^- + HCO_3^-$$

The decomposition in acid solution was still more complicated. It could be disentangled if it was carried out in the presence of mercuric chloride, which caught the cyclohexanethiol formed. The following three primary reactions are postulated:

8 a. $C_6H_{11} \cdot SO \cdot CH(COOH)CH_2COOH \rightarrow \underline{C_6H_{11} \cdot SH} + HOCO \cdot CO \cdot CH_2COOH$

8 b. $C_6H_{11} \cdot SO \cdot CH(COOH)CH_2COOH \rightarrow C_6H_{11} \cdot SOH + \underline{HOCOCH = CHCOOH}$

8 c. $C_6H_{11} \cdot SO \cdot CH(COOH)CH_2COOH \rightarrow$
$\rightarrow C_6H_{11} \cdot S \cdot C(= CH_2)COOH + \underline{CO_2} + H_2O.$

The reaction 8 a. is analogous to 5. The oxaloacetic acid was immediately decarboxylated according to the known scheme (compare SIMON (14)):

8 d. $HOCO \cdot CH_2CO \cdot COOH \rightarrow \underline{CH_3COCOOH} + \underline{CO_2}.$

The sulphenic acid formed in 8 b. was disproportionated:

8 e. $2\,C_6H_{11} \cdot SOH \rightarrow \underline{C_6H_{11} \cdot SH} + C_6H_{11} \cdot SO_2H$

followed by:

8 f. $C_6H_{11} \cdot SOH + C_6H_{11} \cdot SO_2H \rightarrow \underline{C_6H_{11} \cdot S \cdot SO_2 \cdot C_6H_{11}} + H_2O$

8 g. $C_6H_{11} \cdot SOH + C_6H_{11} \cdot SH \rightarrow C_6H_{11} \cdot SS \cdot C_6H_{11} + H_2O.$

Cyclohexyl cyclohexanethiolsulphonate, formula 8 f., is a crystalline compound, which was also prepared by oxidation of dicyclohexyl disulphide with hydrogen peroxide. The formulation of the compound as a disulphoxide now seems obsolete (compare 15). The reaction 8 g. is of less, if any, importance in the presence of mercuric chloride. The cyclohexanesulphinic acid formed according to 8 e. was added to the 1-cyclohexylthioacrylic acid in formula 8 c., yielding 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid:

$$8\,\text{h.}\quad C_6H_{11}\cdot SO_2H + CH_2 = C(S\cdot C_6H_{11})COOH \rightarrow$$
$$\rightarrow \underline{C_6H_{11}\cdot SO_2\cdot CH_2CH(S\cdot C_6H_{11})COOH}.$$

The possibility of this reaction and the structure of the reactants are proved in another work (13). Finally there was formed a sulphur-containing acid, that was isolated as the crystalline S-benzyl-*iso*-thiuronium salt. The empirical composition corresponded to an addition product of pyruvic acid and 1-cyclohexylthioacrylic acid, but owing to the small yield it was not further investigated. The compounds under-lined are those isolated in the experiment. The yields were estimated and found fairly well compatible with the scheme presented. There was no indication of the formation of cyclohexylthiofumaric acid, so it was not considered to be an intermediate, though under similar conditions it was decomposed with formation of several of the above-mentioned compounds (see 13). FITGER (16) made similar experiments with ethylsulphinylsuccinic acid, which on heating with water yielded an ethylthioacrylic acid, by him considered to be the 2-derivative, because the properties of similar crotonic acid derivatives, described by AUTENRIETH (17), pointed in this direction. In the light of the present investigation it seems almost certain, that this assumption was erroneous, and the compound isolated, 1-ethylthioacrylic acid.

**Cyclohexylsulphinimines.** Sulphinimines were first described by MANN and POPE (18). In the case of the indifferent cyclohexyl sulphides it was found most suitable to add an aqueous solution of the chloramine used (benzenesulphonsodiochloroamide, usually named chloramine-B) to a solution of the sulphide in acetone. The sulphinimines were obtained as crystalline products in excellent yields. The experiments with cyclohexylthio-carboxylic acids were carried out in close analogy with those of TANANGER (19). With cyclohexylthioacetic and 2-cyclohexylthiopropionic acid the corresponding sulphinimines were formed, but they were unstable and could not be brought into crystalline form. With cyclohexylthiosuccinic acid the only product was cyclohexylsulphinylsuccinic acid.

**Cyclohexyl sulphones.** The indifferent sulphones were prepared by oxidation of the sulphides in warm glacial acetic acid with excess of 30 % hydrogen peroxide. Cyclohexyl methyl sulphone melted at room temperature, the others had higher melting points. The cyclohexylsulphonyl-carboxylic acids were prepared as the foregoing ones or by the oxidation of neutral, aqueous solutions of the sodium salts of the cyclohexylthio-carboxylic acids with potassium permanganate. The acetic acid derivative was stable in aqueous solution of acid, neutral, or alkaline reaction. 2-Cyclohexylsulphonylpropionic acid and cyclohexylsulphonylsuccinic acid were decomposed in alkaline solution to salts of cyclohexanesulphinic acid and acrylic resp. fumaric acid:

9. $C_6H_{11} \cdot SO_2 \cdot CH_2CH_2COO^- + OH^- \rightarrow C_6H_{11} \cdot SO_2^- + CH_2 = CHCOO^- + H_2O.$

This reaction is an example of those comprised by STUFFER's rule (20).

**Cyclohexanesulphinic acid.** This acid was most conveniently prepared in the way described from cyclohexylsulphonylsuccinic acid, following the method of HOLMBERG (11). In the air it rapidly deteriorated, but kept in a sealed tube it remained unchanged. If air was excluded it was not decomposed by acid or alkali in aqueous solution. The acid may be characterised as its mercurichloride or S-benzyl-*iso*-thiuronium salt. With bromine it yielded, as expected (see for example SUTER (21) p. 513), cyclohexanesulphonylbromide. The reaction between sodium cyclohexanesulphinate and chloramine-B was also investigated. The results obtained are most easily accounted for by the following scheme:

10 a. $C_6H_{11} \cdot SO_2^- + C_6H_5SO_2NCl^- + 2\,H_2O \rightarrow$
$\rightarrow C_6H_{11} \cdot SO_2Cl + C_6H_5SO_2NH_2 + 2\,OH^-$

10 b. $C_6H_{11} \cdot SO_2^- + C_6H_5SO_2NCl^- + H_2O \rightarrow C_6H_{11}SO_3^- + C_6H_5SO_2NH_2 + Cl^-$

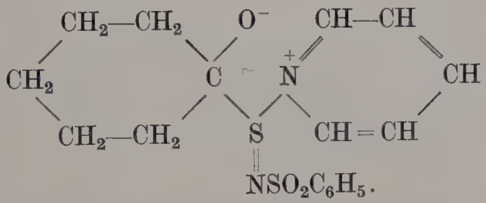
10 c. $C_6H_{11} \cdot SO_2^- + C_6H_5SO_2NCl^- \rightarrow C_6H_{11} \cdot SO_2 \cdot N^- \cdot SO_2 \cdot C_6H_5 + Cl^-.$

During the reaction the mixture turned alkaline and the reaction was retarded. By neutralising by and by, the reaction came to a sharp end point. It followed scheme 10 a., 10 b., and 10 c. to about 65, 25, and 10 %.

HOLMBERG, in a paper on toluene-ω-sulphinic acid (11), made some experiments on the reaction between that acid and unsaturated carboxylic acids. Some similar experiments have now been made on the addition of cyclohexanesulphinic acid to acrylic and maleic acid. In accordance with HOLMBERG's findings there was formed 2-cyclohexylsulphonylpropionic and cyclohexylsulphonylsuccinic acid. In the former case some crude experiments were made as to the reaction velocity. As in the case of toluene-ω-sulphinic acid the velocity passed through a flat maximum with increasing hydrogen ion concentration. With cyclohexene and cyclohexane- or toluene-ω-sulphinic acid there could not be isolated any addition products.

**Some reactions with chloramine.** CLARKE, KENYON, and PHILLIPS (22) showed that chloramine-T with ethanethiol yields ethyl-*p*-toluenesulphonimidosulphine-*p*-toluenesulphonylimine. Compounds of this constitution, by TANANGER (19), are more conveniently considered as amidines of sulphinic acids. ALEXANDER and MCCOMBIE (23) found that such compounds were also formed in the reaction between disulphides and chloramine, but the reaction mechanism postulated by them is obviously not correct, as it is stoichiometrically impossible. ("The sulphide molecule ruptures at the S–S linking and each half reacts as a mercaptan.") BULMER and MANN (24) on the contrary showed, that the reaction with thiols very probably proceeds via the disulphides. This was confirmed and the reaction used to prepare dicyclohexyl disulphide. In his above-mentioned paper TANANGER made some experiments with dibenzyl disulphide and chloramine-B and investigated the reaction more closely than previous authors. However, it still is all but clear, so the present author considered it worth while to study it further in the case of dicyclohexyl disulphide and chloramine-B. Previous authors have neglected the fact, that in the course of the reaction the mixture turns acid, and that the chloramine then immediately disproportionates into sulphonamide and dichloramine. The latter reacts more slowly, if at all, and so no sharp end point is reached in the reaction. It was therefore carried out in pyridine-water or in acetone-water, the acid in the latter case being neutralised by and by with alkali. It was found that cyclohexyl thiolacetate with chloramine-B in pyridine-water yielded much the same products as the disulphide. For comparison cyclohexyl thiolbenzoate and cyclohexyl cyclohexanethiolsulphonate were also reacted with chloramine-B.

In acetone-water dicyclohexyl disulphide and chloramine-B yielded as a main product bisbenzenesulphonylamidine of cyclohexanesulphinic acid. As by-products there were formed benzenesulphonamide, sulphuric acid, cyclohexene (probably), sulphur, cyclohexanesulphonylbenzenesulphonamide, and an acid of unknown constitution, obtained as a sticky mass, and in warm, alkaline solution yielding cyclohexanone, benzenesulphonamide, and sulphur dioxide. In pyridine-water the same main product was obtained. Cyclohexene was not sought for in this experiment, and the three last-mentioned by-products not obtained. Instead there was formed cyclohexanesulphonic acid and a crystalline product, which was insoluble and unaffected by N sodium hydroxide, but soluble in conc. hydrochloric acid. On heating this solution the substance was decomposed to cyclohexanone, benzenesulphonamide, pyridine, and sulphur dioxide. The properties and composition of the compound are best accounted for by the following formula, which considers it to be an amidinium derivative of 1-hydroxy-cyclohexanesulphinic acid.



Stoichiometrically possible is also the formulation of the compound as a pyridinium salt of the benzenesulphonimide of cyclohexenesulphinic acid, $C_6H_9 \cdot SO \cdot N^- \cdot SO_2C_6H_5$, $C_5H_5NH^+$.

In the reaction between cyclohexyl thiolacetate and chloramine-B the main products were bisbenzenesulphonylamidine of cyclohexanesulphinic acid, acetylbenzenesulphonamide, and acetic acid. By-products were benzenesulphonamide, sulphuric acid, cyclohexene, and the above-mentioned amidinium derivative of 1-hydroxy-cyclohexanesulphinic acid, which in this case was formed only in minute amounts.

Cyclohexyl thiolbenzoate reacted more slowly with chloramine-B, so the reaction could not be investigated so closely. It is analogous to the foregoing one, the following products being identified: bisbenzenesulphonylamidine of cyclohexanesulphinic acid (only a minute amount), benzoylbenzenesulphonamide, benzoic acid, benzenesulphonamide, and sulphuric acid.

The investigation of these complicated reactions was obscured by side-reactions between chloramine and the solvents used. There was formed benzenesulphonamide and chlorination products (and their hydrolysis products) of the solvents. It was not possible to isolate and characterise any such compounds. However, if correction was made for the side-reactions it was possible to summarise the results fairly well in the following gross formulas. $R = C_6H_{11}$, $Ph = C_6H_5$.

Dicyclohexyl disulphide and chloramine-B in acetone-water:

11 a. $$RSSR + 3.38\ PhSO_2NCl^- + 1.34\ H_2O \rightarrow 1.25\ RS(:NSO_2Ph)NHSO_2Ph + 0.25\ R \cdot SO_2 \cdot NH \cdot SO_2 \cdot Ph + 0.21\ H_2SO_4 + 0.29\ S + 0.50\ C_6H_{10} + 0.63\ PhSO_2NH_2 + 3.38\ Cl^-.$$

The unidentified acid is assumed to have the same empirical composition as $RSO_2NHSO_2Ph$.

Dicyclohexyl disulphide and chloramine-B in pyridine-water:

11 b. $$RSSR + 3.83\ PhSO_2NCl^- + 1.61\ H_2O + 0.20\ C_5H_5N \rightarrow 1.41\ RS(:NSO_2Ph)NHSO_2Ph + 0.20\ C_6H_{10}(O^-)S(N^+C_5H_5):NSO_2Ph + 0.15\ RSO_3H + 0.24\ H_2SO_4 + 0.81\ PhSO_2NH_2 + 3.83\ Cl^-.$$

Cyclohexyl thiolacetate and chloramine-B in pyridine-water:

11 c. $$RS \cdot COCH_3 + 2.50\ PhSO_2NCl^- + 1.51\ H_2O \rightarrow 0.75\ RS(:NSO_2Ph)NHSO_2Ph + 0.25\ C_6H_{10} + 0.25\ H_2SO_4 + 0.49\ CH_3CO \cdot NH \cdot SO_2Ph + 0.51\ CH_3COOH + 0.51\ PhSO_2NH_2 + 2.50\ Cl\ .$$

It seems not possible to split these formulas into elementary reactions in an unambiguous way. The following scheme therefore is only an illustrative suggestion.

It is assumed, that the initial reaction is a splitting of the disulphide molecule, analogous to the reaction with halogens (12):

12 a. $$RSSR + PhSO_2NCl^- \rightarrow RS \cdot NSO_2Ph^- + RSCl$$

followed by:

12 b. $$RS \cdot NSO_2Ph^- + PhSO_2NCl^- \rightarrow RS(NSO_2Ph)_2^- + Cl^-.$$

The cyclohexanesulphenyl chloride may react in three ways:

12 ca. $$RSCl + H_2O \rightarrow C_6H_{10} + H_2SO + HCl$$

12 cb. $$RSCl + 3\ H_2O + 2\ PhSO_2NCl^- \rightarrow RSO_3H + 2\ PhSO_2NH_2 + 2\ Cl^- + HCl$$

12 cc. $$RSCl + PhSO_2NCl^- \rightarrow RS(Cl):NSO_2Ph + Cl^-.$$

The intermediate compound $H_2SO$ in acetone-water partly decomposes to sulphur, and is partly oxidised by chloramine to sulphuric acid. In pyridine-water this reaction predominates:

12 da. $$H_2SO \rightarrow H_2O + S$$

12 db. $$H_2SO + 3\ PhSO_2NCl^- + 3\ H_2O \rightarrow H_2SO_4 + 3\ PhSO_2NH_2 + 3\ Cl^-.$$

The reaction 12 cb. is of no importance in acetone-water. Reaction 12 cc. may account for the formation of the compound $C_6H_5CH_2 \cdot S(OC_2H_5) : NSO_2C_6H_5$ in the reaction of dibenzyl disulphide with chloramine-B in ethanol, studied by TANANGER (19). In this case the postulated benzenesulphonimide of cyclohexanesulphinyl chloride reacts in three ways:

12 ea. $RSClNSO_2Ph + PhSO_2NH_2 \rightarrow RS(:NSO_2Ph)NHSO_2Ph + HCl$

12 eb. $RSClNSO_2Ph + PhSO_2NCl^- + 2\ H_2O \rightarrow$
$\rightarrow RSO_2 \cdot NH \cdot SO_2Ph + PhSO_2NH_2 + Cl^- + HCl$

12 ec. $RSClNSO_2Ph + C_5H_5N \rightarrow RS(N^+C_5H_5)NSO_2Ph + Cl^-$ followed by
$RS(N^+C_5H_5)NSO_2Ph + PhSO_2NCl^- + H_2O \rightarrow PhSO_2NH_2 + HCl +$
$+ C_6H_{10}(O^-)S(N^+C_5H_5) : NSO_2Ph$.

Reaction 12 ea. explains why there is formed more than one molecule amidine per molecule disulphide. Reaction 12 eb. is of importance in acetone-water only, 12 ec. in pyridine-water.

The unsymmetrical cyclohexyl thiolacetate molecule may be split in two ways:

13 a. $RS \cdot COCH_3 + PhSO_2NCl^- \rightarrow RS \cdot N^- \cdot SO_2Ph + CH_3COCl$ followed by
$CH_3COCl + H_2O \rightarrow CH_3COOH + HCl$

13 b. $RS \cdot COCH_3 + PhSO_2NCl^- \rightarrow RSCl + CH_3CO \cdot N^- \cdot SO_2Ph$.

The reaction then proceeds analogously to the foregoing one.

It is easily possible to combine these elementary reactions to the gross formulas given above.

The constitution of the so-called disulphoxides has been extensively debated in the literature (e.g. 15, 25). They have been considered as true disulphoxides (A), as mixed anhydrides of sulphenic and sulphinic acids (B), and as esters of thiolsulphonic acids (C), the last-mentioned formulation also (25) having been interpreted as representing sulphenyl sulphinates.

(A) $R \cdot SO \cdot SO \cdot R$ (B) $R \cdot S \cdot O \cdot SO \cdot R$ (C) $R \cdot S \cdot SO_2 \cdot R$.

In pyridine-water the cyclohexyl compound reacted slowly with chloramine-B in accordance with the thiolsulphonate formula (C). In the reaction there was isolated, besides unreacted material and benzenesulphonamide, bisbenzenesulphonylamidine of cyclohexanesulphinic acid (only minute amounts), cyclohexanesulphonylbenzenesulphonamide, and sulphuric acid. The reaction thus proceeds in analogy to the reaction between chloramine-B and cyclohexyl thiolacetate:

14. $R \cdot SO_2 \cdot S \cdot R + PhSO_2NCl^- \rightarrow RSO_2 \cdot N^- \cdot SO_2Ph + RSCl$.

The cyclohexanesulphenyl chloride then reacts in the way described above.

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The nomenclature of organic sulphur compounds is in a rather chaotic state. Though it leads to inconsistencies with the present author's previous papers it was considered most convenient to adopt the British practice as it is presented by MITCHELL (26), with the main exception that the prefix cyclo- is not italicised.

## II. Cyclohexylsulphonium compounds

### 1. Indifferent cyclohexylsulphonium salts

### a. Cyclohexyldimethylsulphonium iodide

A mixture of 1.3 g of cyclohexyl methyl sulphide and 1.5 g of methyl iodide almost immediately became turbid. The next day it was partly solid. After four days the hard, crystalline cake was crushed and stirred with 10 ml of ethyl acetate. The following day the crystal powder was sucked off, and yielded 2.6 g of m.p. 98° (efferv.). It was recrystallised from 7 ml of methanol by the addition of 25 ml of ethyl acetate and 50 ml of ether. The precipitate formed consisted of 2.0 g of microscopic prisms, which after drying over phosphorus pentoxide in vacuo had m.p. 105—106°. Borsche and Lange (27) report m.p. 102°. The product was somewhat hygroscopic. Titration according to Volhard gave the equiv. 270.8, calc. for $C_6H_{11} \cdot S(CH_3)_2I$ 272.2. The compound was also prepared in ethyl acetate solution, but then the reaction was much slower.

The stability of the iodide was investigated by distilling its solution in N-HCl, water, and N-NaOH with steam. In no case the decomposition proceeded so rapidly, that it was found worth while to investigate it further. When the compound was heated with ethanolic (95 % ethanol if not otherwise stated) potassium hydroxide it was rapidly decomposed. A solution of 2.7 g of cyclohexyldimethylsulphonium iodide and 0.6 g of potassium hydroxide in 10 ml of ethanol was refluxed on a water bath for one hour. The reflux condenser was connected with a wash bottle containing a solution of 3 g of mercuric chloride in 60 ml of water. There was formed a small amount of a voluminous white precipitate, which sensibly increased when the gases in the reaction vessel were aspirated through the wash bottle after the completion of the reaction. There was obtained 0.2 g of material of m.p. 157—159°, thus identified as the complex of dimethyl sulphide with mercuric chloride (see p. 188).

The ethanolic solution in the reaction vessel was neutralised with 0.7 ml of 2 N-HCl and treated with 50 ml of water. The emulsion formed was extracted with ether, the ether evaporated, and the residue, 1.0 g, treated with a warm solution of 2 g of mercuric chloride in 15 ml of ethanol. On cooling down the solution precipitated 2.25 g of glistening crystal needles of m.p. 89—92°, thus identified as the complex compound $C_6H_{11} \cdot S \cdot CH_3$, $HgCl_2$ described previously (1).

### b. Dicyclohexylmethylsulphonium iodide

This compound was prepared analogously to the foregoing one. From 2.0 g of dicyclohexyl sulphide and 1.5 g of methyl iodide there resulted, after five days, 1.4 g of crystal powder of m.p. 110—112°. It was recrystallised from 5 ml of ethanol by the addition of 5 ml of ethyl acetate and 5 ml of ether. The yield was 0.7 g of colourless needles of m.p. 111—113° (efferv.). Meyer and Hohenemser (28) report the m.p. 110.5°. In this case too the reaction in ethyl acetate was slower. The equiv. was found to be 343.1, calculated for $(C_6H_{11})_2CH_3SI$ 340.3.

The stability of the compound was investigated as above. In aqueous solutions no appreciable decomposition was observed, but in ethanolic potassium hydroxide it was rapidly decomposed. A solution of 1.7 g of the sulphonium salt and 0.3 g of potassium hydroxide in 5 ml of ethanol was refluxed for one hour. It was then neutralised with 0.1 ml of 2 N-HCl. A small amount was distilled off on a water bath and the cyclohexene containing distillate in the way described on p. 186 treated with thiolacetic acid, finally yielding 0.03 g of chloromercury cyclohexyl mercaptide of equiv. 350. In the iodimetric titration of the mercaptide it was found advantageous to use glacial acetic acid instead of ethanolic hydrogen chloride, previously employed (1).

The distillation was continued in a water bath as long as distillate passed over. This was diluted with water and the emulsion formed extracted with ether. Evaporation of the ether left a small oily residue, which was treated with a solution of 0.3 g of mercuric chloride in 5 ml of ethanol, to yield 0.15 g of glistening needles of the complex $C_6H_{11} \cdot S \cdot CH_3$, $HgCl_2$, m.p. and mixed m.p. 88—91°.

The residue from the distillation was treated with water and ether and the mixture decolourised with aqueous sulphur dioxide. The ethereal phase was evaporated, and the oily residue treated with a solution of 1 g of mercuric chloride in 15 ml of warm ethanol. There resulted 1.05 g of soft needles of m.p. 133—135°, on admixture with the complex $(C_6H_{11})_2S$, $HgCl_2$ m.p. 135—137°, and with $C_6H_{11} \cdot S \cdot CH_3$, $2\,HgCl_2$ m.p. 100—115° (compare 1).

## 2. Cyclohexylcarboxyalkylsulphonium salts and thetins

### a. Cyclohexylmethylcarboxymethylsulphonium salts

**Methylation of cyclohexylthioacetic acid.** HOLMBERG (3), in an extensive investigation studied the reaction between alkylthio- and arylthio-acetic acids and dimethyl sulphate. Similar experiments have now been made with cyclohexylthioacetic acid. Each time 1/100 mole of cyclohexylthioacetic acid, 2/100 mole of dimethyl sulphate, and 20 ml of water were used. At the beginning of the experiments various amounts of sodium hydroxide (0, 1/100, or 2/100 mole) were added and the mixtures mechanically agitated until no change was observed. After acidification (if necessary) they were extracted with ether and the ether neutralised. The formation of sulphonium salt was then calculated.

In the first experiment 1.7 g of cyclohexylthioacetic acid, 2 ml of dimethyl sulphate, and 20 ml of water were stirred for 6 hours. A small amount of oil of disagreeable odour at the bottom was not significantly diminished after another 4 hours stirring. Ether then extracted acids neutralised with 0.85 ml of N-NaOH, corresponding to 91.5 % sulphonium salt formation.

In the second experiment a neutral solution of 1.7 g of cyclohexylthioacetic acid, 9.65 ml of N-NaOH, and 10 ml of water was stirred with 2 ml of dimethyl sulphate. The mixture, which almost immediately turned acid, was stirred for 6.5 hours. As in the foregoing experiment there was a small amount of oil. The next day the mixture was neutralised with 7.15 ml of N-NaOH, made clear with ether, and acidified with 5 ml of 5 N-HCl. Ether extracted acid material neutralised with 0.50 ml of N-NaOH, corresponding to 95 % sulphonium salt formation.

Finally, in a third experiment, an alkaline solution of 1.7 g of cyclohexylthioacetic acid in 19.6 ml of N-NaOH was stirred with 2 ml of dimethyl sulphate. After slight emission of heat the mixture became clear and neutral in 15 minutes. After 25 minutes it was acidified with 5 ml of 5 N-HCl, extracted with 15 ml of ether, and the ethereal phase neutralised with 1.00 ml of N-NaOH. The sulphonium salt formation thus amounted to 90 %. In accordance with HOLMBERG's findings the best yield was obtained with an originally neutral mixture.

In an experiment on a somewhat larger scale the properties of the sulphonium compound were investigated. A neutral solution of 7.0 g of cyclohexylthioacetic acid in 41 ml of N-NaOH was treated as above with 4 ml of dimethyl sulphate. After neutralisation some oil drops were extracted with ether and the clear, aqueous solution divided in four equal parts.

The first one was acidified with 4 ml of 5 N-HCl, the turbid solution extracted twice with 10 ml of ether, and the ethereal solutions neutralised with 1.15 + + 0.25 = 1.40 ml of N-NaOH. The sulphonium salt formation thus amounted to 86 %. The clear, aqueous solution was added to a warm solution of 15 g of mercuric chloride in 150 ml of water. The next day there was obtained 8.1 g of small crystals, m.p. 149—152° (decomp.), equiv. 1891 (a specimen was dissolved in aqueous potassium bromide and titrated with 0.1 N-NaOH using phenolphthalein). Recrystallisation from 50 ml of 0.1 N-HCl yielded 3.5 g of a product of m.p. about 150° (decomp.) and equiv. 1867. The mother liquor on free evaporation afforded another 4.9 g that melted as the foregoing product and had the equiv. 1858. The last-mentioned was analysed.

For $C_6H_{11} \cdot S(Cl)(CH_3)CH_2COOH,\ 6\,HgCl_2 = C_9H_{17}O_2Cl_{13}SHg_6$ (1853.89):

|  | Cl | S | Hg |  | equiv. |
|---|---|---|---|---|---|
| calc.: | 24.86 | 1.73 | 64.93 % |  | 1854 |
| found: | 24.78 | 1.73 | 65.33 » |  | 1858 |

Analogous compounds were extensively investigated by STRÖMHOLM (29).

The second part of the above-mentioned sulphonium salt solution was acidified with 5 ml of 2 N-HCl and heated for two hours in a water bath in a distilling flask equipped with a cooled receiver. In the receiver there condensed a few drops smelling of cyclohexene. After cooling down the turbid solution in the distilling flask was extracted twice with 10 ml of ether. The extract was neutralised with 1.9 + 0.3 = 2.2 ml of N-NaOH. The decomposition with formation of acid material thus corresponded to 8 %.

The third neutral part was heated as in the foregoing experiment. There was practically no distillate and no production of acid material.

The fourth part finally was made alkaline with 10 ml of N-NaOH and then treated as before. A small amount of oily distillate, with an odour of cyclohexyl methyl sulphide, was obtained. The alkaline solution, after acidification, was extracted as above. The extract was neutralised with 2.2 ml of N-NaOH, corresponding to a decomposition of 8 %.

**Cyclohexylmethylcarboxymethylsulphonium bromide.** When 4.7 g of cyclohexyl methyl sulphide was treated with 5.0 g of bromoacetic acid there was formed a homogeneous mixture, that soon became turbid and separated a viscous bottom layer. By the following day this began to crystallise. After ten days the almost solid mass was dried on porous tile. It was then digested with ethyl acetate and sucked off. The yield was 5.0 g of m.p. 111—113° (efferv.).

For $C_6H_{11} \cdot S(Br)(CH_3)CH_2COOH = C_9H_{17}O_2BrS$ (269.21):

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| calc.: | C | 40.15 | H | 6.37 | Br | 29.69 | S | 11.91 | equiv. 269.2 |
| found: | » | 40.09 | » | 6.47 | » | 29.71 | » | 11.97 | » 270.7 |

The compound could also be prepared in ethyl acetate solution, or in aqueous solution. In the former case, the sulphonium salt formed, separated by and by as crystal needles. In the latter case, the unhomogeneous mixture on being warmed on a water bath, soon became homogeneous and on evaporation yielded the sulphonium salt.

An attempt was made to prepare the corresponding iodide by methylation of cyclohexylthioacetic acid with methyl iodide, but no crystalline product was isolated from the dark, sticky reaction product.

Cyclohexylmethylcarboxymethylsulphonium bromide was rapidly decomposed in alkaline, ethanolic medium. A solution of 2.7 g of the sulphonium compound and 1.2 g of potassium hydroxide in 10 ml of ethanol was refluxed for one hour on a water bath. It was then neutralised with 2 drops of 2 N-HCl. A faint odour of dimethyl sulphide was observed, but when the gases in the reaction vessel were aspirated through aqueous mercuric chloride only a minute amount of precipitate resulted. The reaction mixture was distilled off on a water bath. The first ml was treated with thiolacetic acid and in the way described on p. 186 yielded 0.3 g of chloromercury cyclohexyl mercaptide of equiv. 347.5. The main portion of the distillate on dilution with water afforded a small amount of an oil with an odour of cyclohexyl methyl sulphide. The partly crystalline residue was treated with water and ether, the ethereal layer evaporated and the oily residue treated with a solution of 0.5 g of mercuric chloride in 10 ml of warm ethanol. There resulted 0.35 g of glistening needles of the complex $C_6H_{11} \cdot S \cdot CH_3$, $HgCl_2$, m.p. 89—91°.

Acidification of the aqueous layer with 2.1 ml of 5 N-HCl gave an emulsion and some carbon dioxide was evolved. The mixture was extracted three times with 15 ml of ether, and the extracts neutralised with $5.4 + 0.7 + 0.2 = 6.3$ ml of N-NaOH. Acidification of the neutral, aqueous solution precipitated some oil drops overnight, which were filtered off, and taken up in ether. This was evaporated, the residue dissolved in 5 ml of glacial acetic acid, oxidised with 1 ml of 30 % hydrogen peroxide, and the solution finally freely evaporated. Seeding with cyclohexylsulphonylacetic acid (see p. 206) induced crystallisation. The crystals were drained on porous tile and recrystallised from ether-light petroleum (b.p. 40—60° if not otherwise stated). A small amount of almost pure cyclohexylsulphonylacetic acid resulted, m.p. 77—80°, equiv. 205, calc. 206.3.

The filtrate from the oil drops was extracted twice with ether and the extracts neutralised with $1.45 + 0.40$ ml of N-NaOH. The neutral aqueous solution was treated with a solution of 1.6 g of mercuric chloride in 45 ml of water. A crust of 0.4 g of poorly developed crystals separated overnight, that did not melt below 200°, but got dark above 160°. They contained 64.28 % Hg and

5.18 % S, whereas for the compound $CH_3 \cdot S \cdot CH_2COOHgCl, HgCl_2$ described by HELLSTRÖM and HOLMBERG (30) is calculated 65.48 and 5.23 % resp.

The aqueous solution, obtained after the extraction of methylthio- and cyclohexylthio-acetic acid, was evaporated to dryness in vacuo over sulphuric acid. The crystalline, sticky residue was extracted twice with 15 ml of acetone, the acetone evaporated, and the residue dried as before. A radiant, hygroscopic, crystalline mass resulted, which was recrystallised from 2 ml of acetone. A minute amount of hard, very hygroscopic, glistening crystals of m.p. 95—98° were obtained and consisted probably of cyclohexyldimethylsulphonium bromide or chloride. According to BORSCHE and LANGE (27) the chloride is hygroscopic.

### b. Cyclohexylmethylthetin

Several attempts were made to prepare this thetin, but no pure product was produced, presumably owing to its instability. In the most successful experiment a solution of 2.7 g of cyclohexylmethylcarboxymethylsulphonium bromide in 8 ml of water was added to a suspension of 1.2 g of silver oxide in 5 ml of water and the mixture stirred. After half an hour the silver bromide formed was filtered off and the filtrate evaporated to dryness in vacuo over phosphorus pentoxide. A sticky crystalline mass resulted, which softened and slowly effervesced above 117°. The material was dissolved in 12 ml of absolute ethanol, some flocks filtered off, and the filtrate treated with 75 ml of ether. A crystalline precipitate soon separated, but after two days the crystals had disappeared and a clear solution was left. Free evaporation yielded a residue, from which no crystalline material could be prepared. It was concluded, that the thetin was unstable in the anhydrous solvent. It was not considered worth while to repeat the experiment with greater care, though it may have been possible to isolate a pure preparation.

### c. Cyclohexylmethyl-2-carboxyethylsulphonium iodide

A mixture of 1.9 g of 2-cyclohexylthiopropionic acid and 1.5 g of methyl iodide after half an hour became turbid and afforded a radiant, crystalline mass overnight. After four days the hard crust was crushed and stirred with 10 ml of ethyl acetate. The following day 3.0 g of crystalline material was sucked off, m.p. 107—109° (efferv.). It was recrystallised from 5 ml of ethanol by addition of 5 ml of ethyl acetate and 5 ml of ether. The yield was 1.8 g of rectangular tablets of m.p. 106—109° (efferv.).

For $C_6H_{11} \cdot S(I)(CH_3)CH_2CH_2COOH = C_{10}H_{19}O_2IS$ (330.23):

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| calc.: | C | 36.37 | H | 5.80 | I | 38.43 | S | 9.71 % | equiv. | 330.2 |
| found: | » | 36.50 | » | 5.79 | » | 38.30 | » | 9.66 » | » | 332.7 |

A solution of 9.5 g of 2-cyclohexylthiopropionic acid and 7.5 g of methyl iodide in 30 ml of ethyl acetate in 4 days afforded 5.4 g of cyclohexylmethyl-2-carboxyethylsulphonium iodide of m.p. 109—112° (efferv.).

In warm, aqueous and warm, neutral, aqueous solution the compound was quite stable. In warm, dilute hydrochloric acid some decomposition with formation of cyclohexyl methyl sulphide was noticed. In alkaline solution the decomposition was rapid.

To get some knowledge of the rate of the alkaline decomposition a solution of 0.1859 g of iodide in 2 × 5.56 ml of 0.1000 N-NaOH was left for one hour at about 20°. The turbid solution was then neutralised with 3.57 ml of 0.1012 N-HCl and again made alkaline with 3.61 ml of 0.1000 N-NaOH. After totally 16 hours the oily solution was neutralised with 1.04 ml of 0.1012 N-HCl. The titrations correspond to 35.1 and 81.2 % decomposition.

In a preparative experiment 1.65 g of the iodide was dissolved in 2 × 5.00 ml of N-NaOH. The solution immediately became turbid and smelt of cyclohexyl methyl sulphide. It was then distilled with steam. After five minutes no more oil drops passed over and the solution was clear and neutral. The oil was taken up in ether, this evaporated, and the residue in the way described on p. 202 reacted with chloramine-B. There resulted 1.1 g of cyclohexylmethylsulphine-benzenesulphonylimine, after recrystallisation from xylene, of m.p. 80.5–82.5°, the same as for an authentic specimen.

The solution distilled was acidified with 3 ml of 2 N-HCl and then treated with 35 ml of 0.2 M toluene-ω-sulphinic acid, prepared according to HOLMBERG (11). In two days 0.75 g of soft crystal flakes had separated. Recrystallisation twice from water afforded pure 2-benzylsulphonylpropionic acid of m.p. 176–177° and equiv. 228.7, calc. 228.1, described by HOLMBERG (11), who reports the m.p. 177—178°.

### d. Cyclohexylmethyl-2-propiothetin

Attempts to prepare this compound were unsuccessful. When an aqueous solution of cyclohexylmethyl-2-carboxyethylsulphonium iodide was neutralised with sodium hydroxide and evaporated to dryness in vacuo over phosphorus pentoxide a hygroscopic glass resulted, but no crystalline material. When instead silver oxide was used and the filtrate from the silver iodide precipitate evaporated in the same way, only a non-crystallising syrup was left. The solution in absolute ethanol did not give any precipitate on addition of ether, and free evaporation only left traces of a residue. Obviously the material was unstable in the anhydrous solvent.

### e. Cyclohexylcarboxymethylthetin

The rate of the reaction between the sodium salts of cyclohexylthioacetic acid and bromo- or chloro-acetic acid in aqueous solution was semi-quantitatively investigated (compare HELLSTRÖM (31) and HOLMBERG, SCHJÅNBERG (32)). The solutions were obtained by dissolving the acids in the appropriate amount of N-NaOH. In each experiment 20.0 ml of each solution were mixed and samples of 2 ml after $T$ hours or $t$ minutes withdrawn, Table 1. They were neutralised with $v$ ml of 0.1000 N-NaOH, then titrated according to VOLHARD, and the amount of ionised bromine (chlorine) calculated in %, $\mathfrak{p}$, of that totally present.

In a preparative experiment, 35.5 g of cyclohexylthioacetic acid, 21 g of chloroacetic acid, and 60 g of cryst. sodium carbonate were dissolved in 100 ml of water. The solution was made neutral with 37 ml of N-NaOH, using phenol red. It was kept in a boiling water bath for five hours. After 1.5 hours 5 ml and after another hour 10 ml of N-NaOH were added. Acidification of the cooled solution with 45 ml of 5 N-HCl afforded a crystalline porridge that was

*Table 1*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyclohexylthioacetate and bromoacetate at room temperature[1] | | | | | | | |
| *T* | $1\frac{1}{3}$ | 21 | 46 | 98 | 168 | | |
| *p* | 46.6 | 79.0 | 86.3 | 90.2 | 91.7 | | |
| Cyclohexylthioacetate and bromoacetate in boiling water bath | | | | | | | |
| *t* | 10 | 20 | 40 | 55 | 75 | 115 | |
| *v* | 0.52 | 0.80 | 1.02[2] | 0.03 | 0.05 | 0.10 | |
| *p* | 88.6 | 94.7 | 97.0 | 98.2 | 98.8 | 100 | |
| Cyclohexylthioacetate and chloroacetate in boiling water bath | | | | | | | |
| *t* | 10 | 25 | 45 | 75 | 105 | 165 | 285 |
| *v* | 0.12 | 0.29 | 0.46 | 0.70[3] | 0.17 | 0.40 | 0.75 |
| *p* | 28.6 | 48.3 | 63.5 | 74.6 | 81.7 | 90.4 | 97.8 |

[1] The production of acid material corresponded to about 1 drop of N-NaOH per day for the whole mixture.

[2] The remaining mixture was neutralised with 1.90 ml of N-NaOH.

[3] The remaining mixture was neutralised with 1.15 ml of N-NaOH.

sucked off. The yield was 31.7 g of a product of m.p. 115° (efferv.) and equiv. 226.5. Digestion with 150 ml of ether left 28.4 g undissolved material of m.p. 109° (efferv.) and equiv. 232.9. The product could be further purified by recrystallisation from ethanol, but losses by decomposition were then inevitable. The m.p. of the pure cyclohexylcarboxymethylthetin was 112—113° (efferv.).

For $C_6H_{11} \cdot S^+(CH_2COO^-)CH_2COOH = C_{10}H_{16}O_4S$ (232.29):
calc.: C 51.70 H 6.94 S 13.80 % equiv. 232.3
found: » 51.74 » 6.98 » 13.77 » » 232.6

The stability of the compound on heating in dilute hydrochloric acid, in water, in dilute sodium hydroxide, and on heating without any solvent was investigated.

A solution of 2.3 g of cyclohexylcarboxymethylthetin in 20 ml of N-HCl was heated on a water bath in a distilling flask with a cooled receiver. During the first half-hour a distillate passed over, that did not increase noticeably on further heating. The heating was interrupted after 1.5 hours. The distillate, which consisted of a light top layer and an aqueous bottom layer, was treated with 1 ml of thiolacetic acid. Some heat was soon emitted. When the reaction was over the mixture was extracted with ether, and the ethereal phase neutralised with 8 ml of N-NaOH. Evaporation of the ether yielded a residue, which was dissolved in 25 ml of ethanol together with 1 ml of 5 N-HCl and 2.7 g of mercuric chloride. Heating for 4 hours on a water bath yielded 2.4 g of glistening flakes of chloromercury cyclohexyl mercaptide of equiv. 352.4, calc. 351.3 (compare 1).

The aqueous solution in the distilling flask was extracted 10 times with ether, and the ethereal solutions neutralised with totally 20.8 ml of N-NaOH. The neutral, ethereal solution was dried with anhydr. sodium sulphate and evaporated. The residue, 0.05 g, smelt of cyclohexanol and was oxidised with conc. nitric acid. There resulted 0.05 g of crystals, which after recrystallisation from dilute hydrochloric acid had m.p. 151–152° and equiv. 76. For adipic acid the m.p. is 153–153.5° (33) and the equiv. 73.1.

The neutral, aqueous solution of the extract was acidified with 5 ml of 5 N-HCl and extracted five times with 50 ml of ether. Drying the extract with calcium chloride and evaporation yielded 1.15 g of crystalline material of m.p. 127–129° and equiv. 77.4. Recrystallisation from ethyl acetate–light petroleum gave 0.8 g of carboxymethylthioacetic acid of m.p. 128.5–129.5° and equiv. 76.1, calc. 75.1. The m.p. of this compound is reported (34) as 129°.

When cyclohexylcarboxymethylthetin was heated in aqueous solution much the same products resulted in the same amounts as in the foregoing case. From 2.3 g of thetin there were isolated 2.3 g of chloromercury cyclohexyl mercaptide, 0.1 g of crude adipic acid, and 1.1 g of crude carboxymethylthioacetic acid.

In a third experiment a solution of 2.3 g of the thetin in 20 ml of N-NaOH was heated as above for two hours. Some distillate passed over and yielded only 0.25 g of chloromercury cyclohexyl mercaptide of equiv. 349.9.

The aqueous residue in the distilling flask was neutralised with 7.84 ml of N-HCl and was then freely evaporated nearly to dryness. Acidification with 6 ml of 2 N-HCl precipitated 1.95 g of somewhat impure thetin of m.p. 121–122° (efferv.) and equiv. 246.2. It was washed with ether and water and then had m.p. 108° (efferv.) and equiv. 237.1.

The mother-liquor from the recovered thetin was extracted twice with ether. Evaporation left 0.15 g of crystals, which were recrystallised from ethyl acetate-light petroleum, and yielded 0.1 g of carboxymethylthioacetic acid of m.p. 129–130° and equiv. 76.0.

Preliminary experiments showed, that heating of the thetin to 120° afforded the following products: cyclohexene, dimethyl sulphide, carbon dioxide, and cyclohexylmethyl sulphide. In order to determine the amount of carbon dioxide formed, 0.1332 g of thetin was heated to 120° and the gases formed were aspirated with carbon dioxide free air through four wash bottles totally containing a mixture of 90.6 ml of 0.1000 N-NaOH and 10 ml of 10 % barium chloride. The last bottle remained clear, in the other ones barium carbonate was precipitated. After the reaction the contents were neutralised with 72.3 ml of 0.1000 N-HCl using phenolphthalein. Thus there was formed 1.59 moles of $CO_2$ per mole of thetin. In a preparative experiment 29.5 g of thetin was heated to 120°. The evolution of gases ceased after about half an hour and then the heating was interrupted. The gases were cooled in a receiver and then passed through a wash bottle with 75 ml of 5 % aqueous mercuric chloride. There resulted 1.3 g of a flocky precipitate of m.p. 157–159° which was thus identical with the dimethyl sulphide - mercuric chloride - complex used by CHALLENGER et al. (35) for the identification of dimethyl sulphide. These authors did not analyse their material, but considered it identical with that described and analysed by FARAGHER, MORRELL, and COMAY (36), approximately having the formula $2\,(CH_3)_2S,\ 3\,HgCl_2$ and m.p. (on rapid heating) 151°. This compound was formed in ethanolic solution and recrystallised from benzene. FARAGHER et al. counted with the possibility that their material was a mixture of $(CH_3)_2S$, $HgCl_2$ and $(CH_3)_2S$, $2\,HgCl_2$. The author therefore considered it necessary to analyse his preparation which was found to correspond to the formula $(CH_3)_2S$, $2\,HgCl_2$, somewhat deficient in $HgCl_2$.

For $(CH_3)_2S,\ 2\,HgCl_2 = C_2H_6Cl_4SHg_2$ (605.19):

| | | | | | | |
|---|---|---|---|---|---|---|
| calc.: | Cl | 23.44 | S | 5.30 | Hg | 66.30 % |
| found: | » | 23.10 | » | 5.53 | » | 65.80 » |

In the cooled receiver 0.5 g of liquid had condensed. Treatment with thiolacetic acid in the usual way yielded 0.8 g of chloromercury cyclohexyl mercaptide of equiv. 345.3.

The residue in the reaction vessel consisted of 19.6 g of a brown liquid with a small amount of solid material. It was mixed with 50 ml of ether and filtered, some flocky material being undissolved. This was dried in vacuo over phosphorus pentoxide and consisted of 0.5 g of a hygroscopic, brown powder, that did not change below 235°. With acids carbon dioxide was evolved. It contained 18 % ash and (counted on ash-free subst.) 55.29 % C, 6.86 % H, and 13.20 % S.

The ethereal filtrate was washed three times with 15 ml of water and then dried with calcium chloride. The washings on evaporation in vacuo left 0.6 g of a brown syrup, not further investigated. The ethereal solution was evaporated and the residue distilled. After some light-boiling material (ether) the following fractions were collected: at 53–58°/10 mm 9.95 g of a colourless oil, up to 84°/1 mm 1.0 g, and at 86–91°/1 mm 3.02 g of a colourless oil. The dark, viscous residue weighed 2.15 g. The first fraction consisted of cyclohexyl methyl sulphide, identified as the complex $C_6H_{11}\cdot S\cdot CH_3$, $HgCl_2$. From 0.65 g of the distillate and 1.4 g of mercuric chloride in 15 ml of warm ethanol 1.4 g of glistening needles of the complex was formed, m.p. 89–92°. The fraction of b.p. 86–91°/1 mm, according to analysis of a product obtained in a preliminary experiment, mainly consisted of methyl cyclohexylthioacetate. It was therefore dissolved in 10 ml of ethanol and treated with 25.00 ml of N-NaOH. After three days no more alkali was consumed and the almost clear solution neutralised with 2.72 ml of 2 N-HCl. The neutral solution was extracted with 30 ml of ether and the ethereal layer treated with a solution of 0.8 g of mercuric chloride in 10 ml of ethanol. There was formed 0.81 g of chloromercury cyclohexyl mercaptide of equiv. 356.5. The aqueous layer was acidified with 5 ml of 5 N-HCl, the emulsion formed was extracted with 30 ml of ether, and this neutralised with 13.9 ml of N-NaOH. The extraction was then repeated four times, removing acids corresponding to $1.9+0.95+0.5+0.45=3.8$ ml of N-NaOH. The extracted solution was then mixed with a warm solution of 0.6 g of 2,4-dinitrophenylhydrazine in 35 ml of 4 N-HCl, almost immediately affording yellow crystal needles. The yield was 0.16 g of the 2,4-dinitrophenylhydrazone of formaldehyde, m.p. 165–166°, unaltered on admixture with an authentic specimen. ALLEN (37) reports the m.p. 155°.

The first of the above-mentioned neutral, aqueous extracts (corresponding to 13.9 ml of N-NaOH) was acidified with 3 ml of 5 N-HCl, the oil formed filtered off, dissolved in ether, this neutralised with 9.0 ml of N-NaOH, the oil reprecipitated and extracted with ether, the ether evaporated, and the residue finally dried at the water pump on a water bath. The yield of colourless oil was 1.5 g, equiv. 174.5, calc. for cyclohexylthioacetic acid 174.3. It was oxidised in the usual way to cyclohexylsulphonylacetic acid (see p. 206), m.p. 81–84°, equiv. 205.9, calc. 206.3.

The filtrate from the oil was added to the second of the above-mentioned neutral, aqueous extracts (corresp. to 3.8 ml of N-NaOH) and acidified with 1 ml of 5 N-HCl. Four extractions with 50 ml of ether and evaporation of the ether yielded an oil, that partly crystallised. It was washed with light petroleum, which left 0.3 g of solid material undissolved. Recrystallisation from ethyl acetate-benzene yielded almost pure carboxymethylthioacetic acid of m.p. 125–127° and equiv. 76.8, calc. 75.1.

The dark residue from the distillation, 2.15 g, was treated in just the same way. In this case too acid products resulted, but they did not crystallise and could not be identified.

### f. Cyclohexylcarboxymethyl-2-carboxyethylsulphonium salts

In preliminary experiments the reaction between 2-cyclohexylthiopropionic acid and bromoacetic acid was carried out in various solvents and without any solvent. It was found, that the reaction proceeded most rapidly in aqueous solution.

A homogeneous mixture of 18.8 g of 2-cyclohexylthiopropionic acid, 13.9 g of bromoacetic acid, and 10 ml of water was left for three days. The green, viscous syrup was then treated with 45 ml of acetone and stirred. A thick crystal porridge was formed. It was sucked off and washed with acetone. The yield of a brilliant, white crystal powder of microscopic needles was 26.1 g. It consisted of pure cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide, which in a sealed capillary melted at 104–107°, in an open one at 116–118°, after softening.

For $C_6H_{11} \cdot S(Br)(CH_2COOH)CH_2CH_2COOH,\ H_2O = C_{11}H_{21}O_5BrS$ (345.26):

| | | | | | | |
|---|---|---|---|---|---|---|
| calc.: | C 38.26 | H 6.13 | Br 23.15 | S 9.29 | $H_2O$ 5.22 % | equiv. 172.6 |
| found: | » 38.44 | » 6.16 | » 23.17 | » 9.26 | » 5.22 » | » 171.8 |

The chloride was prepared analogously, but in this case the reaction was so slow, that the method is of little preparative importance. When a homogeneous mixture of 1.9 g of 2-cyclohexylthiopropionic acid, 0.95 g of chloroacetic acid, and 1 ml of water after one month was stirred with 20 ml of acetone only 0.6 g of crystal powder was obtained, m.p. 129–130° (efferv., open or sealed capillary).

For $C_6H_{11} \cdot S(Cl)(CH_2COOH)CH_2CH_2COOH = C_{11}H_{19}O_4ClS$ (282.78):

| | | | | | |
|---|---|---|---|---|---|
| calc.: | C 46.72 | H 6.77 | Cl 12.54 | S 11.34 % | equiv. 141.4 |
| found: | » 46.95 | » 6.96 | » 12.50 | » 11.26 » | » 141.5 |

The stability of the bromide was investigated on heating in aqueous solution and in aqueous solution containing one and three equivalents of sodium hydroxide.

A solution of 3.5 g of cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide in 20 ml of water was heated for four hours in a water bath contained in a distilling flask with a cooled receiver. The distillate formed consisted of an aqueous bottom layer and a top layer of cyclohexene, which in the usual way was converted to 2.0 g of chloromercury cyclohexyl mercaptide of equiv. 347.3.

The aqueous residue in the distilling flask contained some oil drops. The mixture was extracted 9 times with 30 ml of ether and the extract neutralised with 7.5 + 5.2 + 1.4 + 1.3 + 1.3 + 1.2 + 0.9 + 0.8 + 0.5 = 20.1 ml of N-NaOH. The finally obtained neutral, ethereal solution was dried with anhydr. sodium sulphate and evaporated. The oily residue, 0.05 g, was oxidised with conc. nitric acid to yield adipic acid, recrystallised from dilute HCl, m.p. 149–151°.

The neutral, aqueous solution from the first extract, 7.5 ml, was acidified with 2 ml of 5 N-HCl, some oil drops (0.15 g) filtered off (see below), and the filtrate treated with 25 ml of 0.2 M toluene-$\omega$-sulphinic acid. After some days 0.05 g of crystal powder was sucked off, recrystallised from water and identified as 2-benzylsulphonylpropionic acid of m.p. 176–178° and equiv. 226.

The other neutral, aqueous solutions were taken together, acidified with 3 ml of 5 N-HCl, and extracted three times with 50 ml of ether. Evaporation of the ether afforded 0.7 g of a radiant crystalline mass, which was recrystallised from ethyl acetate-light petroleum. The purified material was identified as 2-carboxymethylthiopropionic acid of m.p. 92–94° and equiv. 82.7, calc. 82.1. LOVÉN (38) reports the m.p. 94°.

The oil mentioned above, 0.15 g, was as usual oxidised to cyclohexylsulphonylacetic acid, m.p. 79–82°.

A solution of 3.5 g of cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide in a mixture of 10 ml of water and 10 ml of N-NaOH was heated as in the foregoing experiment. A drop of aqueous barium hydroxide, held in the vapour, rapidly became turbid, indicating the presence of carbon dioxide. Only a small amount of cyclohexene was condensed in the receiver. It was converted to 0.3 g of chloromercury cyclohexyl mercaptide, equiv. 346.0.

The aqueous residue was brown with an oily bottom layer. It was extracted four times with 50 ml of ether and the extracts neutralised with 13.8 + 2.2 + 0.9 + 0.5 = 17.4 ml of N-NaOH. The neutral, ethereal solution was dried with calcium chloride and evaporated to yield 0.2 g of an oil of a sharp odour. It contained 59.54 % C, 8.54 % H, and 17.15 % S, whereas for $C_6H_{11} \cdot S \cdot CH_2COOCH_3$ is calc. 57.41 % C, 8.57 % H, and 17.03 % S.

The neutral, aqueous solution of the first extract, 13.8 ml, was treated as in the foregoing experiment to yield some oil and 0.75 g of crude 2-benzylsulphonylpropionic acid, after recrystallisation from water of m.p. 176–178°, equiv. 227.6. The oil was oxidised to 1.0 g of crude cyclohexylsulphonylacetic acid, recrystallised of m.p. 78–81° and equiv. 207.9.

The other neutral extract solutions were taken together, acidified and extracted with ether, but it was not possible to prepare any crystalline product from the small amount, 0.15 g, of oily extract obtained.

To get some knowledge of the rate of the alkaline decomposition 0.2562 g of the bromide was dissolved to a neutral solution in 14.68 ml of 0.1012 N-NaOH and made alkaline with 7.34 ml of the same NaOH. After one hour at about 20° the solution was neutralised with 5.76 ml of 0.1012 N-HCl, again treated with 5.76 ml of the NaOH, and finally after a total of 19 hours, neutralised with 2.07 ml of the acid. The titrations correspond to 21.5 and 71.8 % decomposition.

In a preparative experiment a neutral solution of 3.5 g of cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide in 19.7 ml of N-NaOH was made alkaline with further 9.9 ml and heated in a water bath for one hour. The solution was then neutral. Acidification with 10 ml of 5 N-HCl afforded an oil, that was filtered off. The filtrate was treated with 60 ml of 0.2 M toluene-$\omega$-sulphinic acid, which after five days precipitated 1.8 g of 2-benzylsulphonylpropionic acid, recrystallised from water, of m.p. 176–177° and equiv. 229.1.

The oil in the usual way gave 1.5 g of crude cyclohexylsulphonylacetic acid, recrystallised from benzene, of m.p. 80–83°, equiv. 206.0.

### g. Cyclohexyl-2-carboxyethylthetin

An attempt was first made to prepare this compound in the same way as cyclohexylcarboxymethylthetin, i.e. in neutral, aqueous solution of sodium salts of 2-cyclohexylthiopropionic acid and bromoacetic acid. The reaction proceeded at almost the same rate as in that case, but no thetin was isolated, only products, formed by its decomposition, i.e. cyclohexylthioacetic acid and acrylic acid.

However, the thetin could be prepared from its hydrobromide previously described, either by means of ethanolic potassium hydroxide or silver oxide in water. According to the first method a solution of 3.5 g of cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide in 10 ml of ethanol was mixed with 11.3 ml of 0.885 N ethanolic KOH. The next day, 0.95 g of potassium bromide was filtered off and the filtrate freely evaporated. By and by aggregates of crystal needles separated, but also some sticky material. The product, which contained some potassium bromide, was powdered and washed with small amounts of ethanol and water. Finally 0.6 g of bromide free product resulted, m.p. 108° (efferv.), equiv. 248.6. It was further purified by adding 35 ml of acetone to the solution in 5 ml of water. Small crystal rods resulted, m.p. 105° (efferv.).

For $C_6H_{11} \cdot S^+(CH_2COO^-)CH_2CH_2COOH = C_{11}H_{18}O_4S$ (246.31):

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| calc.: | C | 53.64 | H | 7.36 | S | 13.02 % | equiv. | 246.3 |
| found: | » | 54.00 | » | 7.55 | » | 13.07 » | » | 245.0 |

According to the second method a solution of 3.5 g of cyclohexylcarboxymethyl-2-carboxyethylsulphonium bromide was added to a suspension of 1.2 g of silver oxide in 5 ml of water. The mixture was stirred, and filtered after half an hour. The filtrate was treated with 190 ml of acetone, which precipitated 1.25 g of crystal rods, somewhat greyish, of m.p. 114–116° (efferv.). Addition of 100 ml of ether to the mother-liquor yielded a further 0.2 g. The products were dissolved in 6 ml of water and reprecipitated with 40 ml of acetone to yield 0.7 g of almost colourless crystals of m.p. 114–115° (efferv.) and equiv. 248.1.

### h. Sulphonium derivatives of cyclohexylthiosuccinic acid

Attempts to prepare sulphonium compounds derived from cyclohexylthiosuccinic acid were altogether unsuccessful. No reaction was observed between cyclohexylthiosuccinic acid and methyl iodide or bromoacetic acid, or between

2-cyclohexylthiopropionic acid and bromosuccinic acid. In warm, neutral, aqueous solution sodium salts of cyclohexylthiosuccinic acid and bromoacetic acid reacted with each other, though the reaction was slower than that of the cyclohexylthioacetate, but no thetin could be isolated, only decomposition products (cyclohexylthioacetic acid) and unreacted cyclohexylthiosuccinic acid.

## III. Cyclohexyl sulphoxides

### 1. Indifferent cyclohexyl sulphoxides

### a. Cyclohexyl methyl sulphoxide

A solution of 7.6 g of cyclohexyl methyl sulphide in 50 ml of acetone was treated with 5.9 ml of 30 % hydrogen peroxide. No rise of temperature was observed. Free evaporation yielded an almost odourless oil, which was dissolved in 25 ml of water. Ether extracted 0.15 g of an oil, emulsified with water. The aqueous solution was evaporated and dried in vacuo over sulphuric acid and finally over phosphorus pentoxide. The residue, 6.4 g, was destilled at 0.5 mm and gave 5.3 g of a distillate of b.p. 110–120°, which smelt of cyclohexyl methyl sulphide and with 10 ml of water gave an opalescent solution. This was cleared up with ether and then evaporated and dried as above to yield 3.9 g of a very hygroscopic oil, which on cooling with solid carbon dioxide solidified to a crystalline mass, melting at 8–9°. For cyclohexyl methyl sulphoxide BARNARD, FABIAN and KOCH (39) report b.p. 88°/0.05 mm, $n_D^{20}$ 1.5119.

For $C_6H_{11} \cdot SO \cdot CH_3 = C_7H_{14}OS$ (146.24):

calc.: C 57.49 H 9.65 S 21.92 %
found: » 57.32 » 9.59 » 21.98 »
$d_4^{20}$ 1.0689, $n_D^{20}$ 1.5107, mol.refr. 40.97, refr. of the sulphinyl-group 8.64.

The refraction of the sulphinyl-group was calculated using EISENLOHR'S (40) values for the refraction of the other atoms. STRECKER and SPITALER (41) for this group give the refraction 8.59.

### b. Dicyclohexyl sulphoxide

A solution of 2.0 g of dicyclohexyl sulphide in 15 ml of acetone was oxidised with 1 ml of 30 % hydrogen peroxide. Free evaporation gave a viscous residue, that crystallised when scratched. The yield was 2.1 g. Recrystallisation from 35 ml of light petroleum afforded 1.4 g of colourless crystal plates of m.p. 85.5–87°.

For $(C_6H_{11})_2SO = C_{12}H_{22}OS$ (214.36):

calc.: C 67.23 H 10.35 S 14.96 %
found: » 66.98 » 10.31 » 14.91 »

### c. Cyclohexyl phenyl sulphoxide

The preparation was made analogously to the foregoing ones. From 1.9 g of cyclohexyl phenyl sulphide a crude oil resulted, which crystallised when cooled (ice + salt) and scratched. Draining on porous tile yielded 1.9 g of a product of m.p. 53–56°, which was further purified by recrystallisation from light petroleum, m.p. 61–63.5°.

For $C_6H_{11} \cdot SO \cdot C_6H_5 = C_{12}H_{16}OS$ (208.31):

| | | | |
|---|---|---|---|
| calc.: | C 69.19 | H 7.74 | S 15.39 % |
| found: | » 69.07 | » 7.69 | » 15.38 » |

### d. Cyclohexyl benzyl sulphoxide

The preparation was carried out as above. From 2.1 g of cyclohexyl benzyl sulphide 1.4 g of crude sulphoxide resulted, m.p. 80–82°. It was recrystallized from 5 ml of benzene by the addition of 15 ml of light petroleum to yield 1.1 g of small crystal needles of m.p. 84–85°.

For $C_6H_{11} \cdot SO \cdot CH_2C_6H_5 = C_{13}H_{18}OS$ (222.33):

| | | | |
|---|---|---|---|
| calc.: | C 70.22 | H 8.16 | S 14.42 % |
| found: | » 70.19 | » 8.15 | » 14.43 » |

### e. Cyclohexyl cyclohexanethiolsulphonate (“Dicyclohexyl disulphoxide”)

This compound is described here, because it was prepared as were the true sulphoxides. A solution of 2.3 g of dicyclohexyl disulphide in 30 ml of acetone was treated with 2 ml of 30 % hydrogen peroxide. The mixture was freely evaporated. After 3 weeks an oil was left, with a brown bottom layer. Some water was added and after a week crystals resulted. These were drained on porous tile and weighed 0.7 g. Recrystallisation from 1.5 ml of ethanol afforded the pure cyclohexyl cyclohexanethiolsulphonate as small crystal rods of m.p. 37—38°. They were soluble in all the common organic solvents.

For $C_6H_{11} \cdot SO_2 \cdot S . C_6H_{11} = C_{12}H_{22}O_2S_2$ (262.42):

| | | | |
|---|---|---|---|
| calc.: | C 54.92 | H 8.45 | S 24.43 % |
| found: | » 55.09 | » 8.46 | » 24.63 » |

The compound was previously described by VON BRAUN and WEISSBACH (42) as an oil, and by COWIE and GIBSON (43), who report the m.p. 38°.

## 2. Cyclohexylsulphinyl-carboxylic acids

### a. Cyclohexylsulphinylacetic acid

As this substance was rather unstable, special precautions had to be taken in its preparation. A cold solution of 8.7 g of cyclohexylthioacetic acid in 50 ml of acetone was treated with 5 ml of 30 % hydrogen peroxide and then immediately evaporated at the pump at room temperature. After about 4 hours no acetone remained. The thick, oily residue was dried in vacuo over phosphorus pentoxide. It soon crystallised, was powdered, and then dried as before overnight. The product then weighed 9.4 g, melted at 77–80° and had the equiv. 192.5. It was recrystallised from acetone by the addition of light petroleum to yield glistening crystal flakes of m.p. 82–84°. The compound was soluble in water, ethanol, ethyl acetate, and acetone, less so in benzene, ether, and light petroleum.

For $C_6H_{11} \cdot SO \cdot CH_2COOH = C_8H_{14}O_3S$ (190.25):
calc.: C 50.50 H 7.42 S 16.85 % equiv. 190.3
found: » 50.63 » 7.35 » 16.81 » » 190.3

The stability of the compound when heated alone and in warm, aqueous solution of varying acidity and alkalinity was investigated.

When 1.9 g of cyclohexylsulphinylacetic acid was heated for 4 hours in a water bath a melt was formed, which by and by yielded two layers, the upper light-fluid, the lower viscous. On cooling 1.8 g of a very viscous mixture resulted. It was treated with ether. Some undissolved material was dissolved in water. The ethereal solution was extracted twice with water, and the aqueous solution and extracts neutralised with 3.81 ml of N-NaOH, acidified, and treated with a solution of 0.8 g of phenylhydrazine hydrochloride in 5 ml of water. Overnight 0.5 g of yellow-green crystal flakes resulted, which after recrystallisation from acetone melted at 132–135° (decomp.) and had the equiv. 165.7. The equiv. of glyoxylic acid phenylhydrazone is 164.3 and its m.p. reported (44) as 137° (decomp.). The ethereal solution was neutralised with 5.43 ml of N-NaOH (phenolphthalein, sharp end point) and then treated with ethanolic mercuric chloride to yield 0.01 g of chloromercury cyclohexyl mercaptide of equiv. 358. Acidification of the neutral, aqueous solution gave an oil, which on being cooled in the ice-safe solidified to 1.4 g of a crystalline mass of m.p. 43–48°, equiv. 291.3. The substance was very soluble in organic solvents, and was recrystallised with great losses from 2 ml of light petroleum to yield 0.6 g of biscyclohexylthioacetic acid of m.p. 46–48°.

For $(C_6H_{11} \cdot S)_2CHCOOH = C_{14}H_{24}O_2S_2$ (288.45):
calc.: C 58.29 H 8.39 S 22.23 % equiv. 288.5
found: » 58.24 » 8.44 » 22.15 » » 288.3

A solution of 0.95 g of cyclohexylsulphinylacetic acid in 10 ml of water was distilled with steam. Oil drops passed over. They smelt of cyclohexanethiol. During 1.5 hours 300 ml of water distilled over, finally only with traces of oil. The oil was taken up in ether, and the ether treated with ethanolic mercuric chloride to yield 0.65 g of crystal powder of chloromercury cyclohexyl mercaptide of equiv. 350.3. The clear and odourless solution in the distilling vessel was neutralised with 5.00 ml of N-NaOH. It was reacidified and treated with 0.8 g of phenylhydrazine hydrochloride in 20 ml of water to yield 0.55 g of crude glyoxylic acid phenylhydrazone, purified by recrystallisation from ethyl acetate-light petroleum, m.p. 130–131°, equiv. 169.6.

When 0.95 g of the same compound in 10 ml of N-HCl was steam distilled as above, all oil passed over with 100 ml of water in half an hour. The yield of chloromercury cyclohexyl mercaptide was 1.2 g, equiv. 351.8. The solution distilled contained a small amount of an oil, which on seeding with biscyclohexylthioacetic acid solidified, m.p. 43–45°, equiv. 298. The filtrate from this product yielded 0.75 g of glyoxylic acid phenylhydrazone.

Neutral or alkaline solutions of cyclohexylsulphinylacetic acid were practically stable when heated for 4 hours in a water bath. The acid was recovered almost unchanged.

When finally a solution of 0.95 g of cyclohexylsulphinylacetic acid and 1.5 g of mercuric chloride in 20 ml of N-HCl was heated on a water bath for 4 hours a thick porridge of glistening flakes of chloromercury cyclohexyl mercaptide was formed by and by. The yield was 1.75 g and the equiv. 350.8.

Aqueous solutions of sodium cyclohexylsulphinylacetate were prepared by oxidation of aqueous sodium cyclohexylthioacetate with hydrogen peroxide or potassium persulphate. A neutral solution of 1.7 g of cyclohexylthioacetic acid and 1.4 g of cryst. sodium carbonate in 10 ml of water was treated with 1 ml of 30 % hydrogen peroxide. Some heat was emitted. The next day the somewhat turbid solution was cleared with ether, and after acidification treated with 2.7 g of mercuric chloride on a water bath to yield 3.4 g of chloromercury cyclohexyl mercaptide of equiv. 350.6. In the mother-liquor glyoxylic acid was precipitated with phenylhydrazine and yielded 1.45 g of the hydrazone. In another experiment a solution of 1.7 g of cyclohexylthioacetic acid and 4.3 g of cryst. sodium carbonate in 20 ml of water was oxidised with 2.7 g of powdered potassium persulphate. Carbon dioxide was soon evolved and the temperature rose a little. When the evolution of carbon dioxide was over, the solution was neutral. The next day it was worked up as in the foregoing experiment and yielded 3.25 g of mercaptide and 1.3 g of hydrazone.

### b. 2-Cyclohexylsulphinylpropionic acid

A solution of 18.8 g of 2-cyclohexylthiopropionic acid in 100 ml of acetone was oxidised with 10 ml of 30 % hydrogen peroxide. A slight emission of heat was noticed. Free evaporation yielded a syrup overnight, which on being scratched solidified to a moist, crystalline mass. It was dried in vacuo over phosphorus pentoxide and then weighed 20.5 g, softened at 85°, and melted at 104°, and had the equiv. 204.7. Recrystallisation from 100 ml of ethyl acetate afforded 15.7 g of pure 2-cyclohexylsulphinylpropionic acid of m.p. 108–111°. The compound was soluble in water, ethanol, acetone and ethyl acetate, slightly soluble in ether, benzene, and light petroleum.

For $C_6H_{11} \cdot SO \cdot CH_2CH_2COOH = C_9H_{16}O_3S$ (204.28):

| | | | | |
|---|---|---|---|---|
| calc.: | C 52.91 | H 7.90 | S 15.69 % | equiv. 204.3 |
| found: | » 53.00 | » 8.01 | » 15.59 » | » 205.6 |

The stability of the compound was investigated under various conditions.

A solution of 1.0 g of 2-cyclohexylsulphinylpropionic acid in 10 ml of water was heated in a water bath for 4 hours. A small amount, 0.05 g, of ether-soluble oil was formed and filtered off. Reduction with zinc powder in ethanolic hydrogen chloride afforded cyclohexanethiol, which with ethanolic mercuric chloride afforded glistening crystals of the chloromercury mercaptide. The oil thus consisted of dicyclohexyl disulphide. The filtrate from the oil was seeded with 2-cyclohexylsulphinylpropionic acid and yielded 0.4 g of white crystals of m.p. 103–105° and equiv. 205.7. Two portions of 10 ml of ether from the mother-liquor extracted acids corresponding to 0.53 + 0.24 ml of N-NaOH. The neutral solution of the extract was acidified with 1 ml of N-HCl and treated with 5 ml of 0.2 M toluene-ω-sulphinic acid. After 2 days 0.05 g of somewhat impure 2-benzylsulphonylpropionic acid resulted, m.p. 172–174°, equiv. 222. Evaporation of the extracted solution in vacuo over phosphorus pentoxide yielded a further 0.35 g of recovered 2-cyclohexylsulphinylpropionic acid, after recrystallisation of m.p. 103–105°, equiv. 207.0.

Much the same was the result when the acid was heated in N-HCl, or in a solution N with respect to HCl and $HgCl_2$. In the latter case no chloromercury cyclohexyl mercaptide was formed, but some dicyclohexyl disulphide as in the other experiments.

When a neutral solution of 2-cyclohexylsulphinylpropionic acid was heated as above no decomposition was observed.

Finally the decomposition in alkaline solution was investigated. A solution of 2.04 g of 2-cyclohexylsulphinylpropionic acid in 2 × 10.0 ml of N-NaOH was heated in a water bath for 4 hours. Some oil separated. When cool the solution became neutral with 6.8 ml of N-HCl. The oil was taken up in ether, the ether evaporated, and the residue, 0.7 g of oil, reduced to cyclohexanethiol with zinc powder in aqueous-ethanolic hydrogen chloride. The thiol was distilled off with steam, taken up in ether, and the ether finally treated with ethanolic mercuric chloride to yield 1.65 g of chloromercury cyclohexyl mercaptide of equiv. 351.6. The extracted solution was acidified with 5 ml of 5 N-HCl and extracted with 8 × 15 ml of ether. The extracts were neutralised with 7.13 + 1.87 + 1.20 + 0.60 + 0.30 + 0.10 + 0.10 + 0.05 = 11.35 ml of N-NaOH. The neutral extract solution was parted in two equal portions. The one was treated with 25 ml of 0.2 M toluene-ω-sulphinic acid to yield, after 3 days, 0.2 g of crude 2-benzylsulphonylpropionic acid, recrystallised from water to glistening flakes of m.p. 176–177°, equiv. 228.2. The other portion was evaporated to dryness in vacuo over potassium hydroxide to yield 0.85 g of a radiant mass. Extraction with acetone left 0.55 g of a gummy mass undissolved. Free evaporation of the extract afforded 0.3 g of a radiant mass of m.p. 87–100°, equiv. 233.5. Recrystallisation from benzene yielded glistening needles of m.p. 103–106° and equiv. 221.3, for 2-cyclohexylsulphonylpropionic acid (see p. 207) calc. 220.3.

In another experiment half the amount of 2-cyclohexylsulphinylpropionic acid was used. The oil formed was extracted, the aqueous layer neutralised with 2 N-$H_2SO_4$, and then treated with a solution of 0.3 g of mercuric chloride in 3 ml of warm water. A precipitate of 0.5 g of microscopic crystals resulted. It contained 52.56 % Hg, whereas for the chloromercury salt of cyclohexanesulphinic acid is calc. 52.34 % (see p. 207). The mother-liquor as above yielded 0.65 g of crude 2-benzylsulphonylpropionic acid, recrystallised from water, of m.p. 176–178° and equiv. 226.7.

### c. Cyclohexylsulphinylsuccinic acid

This compound was even more unstable than cyclohexylsulphinylacetic acid and it was rather difficult to obtain free from fumaric acid.

The best result occurred in the following way. A solution of 11.6 g of cyclohexylthiosuccinic acid in 40 ml of acetone was cooled to 13° and then treated with 5 ml of 30 % hydrogen peroxide. The temperature rose to 22°, the solution was again cooled to 13°, and then left at room temperature overnight. It was then evaporated in vacuo, finally over phosphorus pentoxide. The yield was 10.8 g of a sticky mass, which was first extracted with 5 ml of acetone, then with 30 ml of light petroleum, to yield 9.8 g of undissolved material of equiv. 120.3. Recrystallisation of 2 g of it from 5 ml of acetone (cooling with ice) afforded 1.0 g of pure cyclohexylsulphinylsuccinic acid. The substance had no definite m.p. It got yellowish above 130°, and was observed to be moist above the solid part in the capillary before it melted at 237–240° (efferv.).

For $C_6H_{11} \cdot SO \cdot CH(COOH)CH_2COOH = C_{10}H_{16}O_5S$ (248.29):

| | | | | |
|---|---|---|---|---|
| calc.: | C 48.37 | H 6.50 | S 12.91 % | equiv. 124.1 |
| found: | » 48.40 | » 6.42 | » 12.96 » | » 123.8 |

From the mother-liquor more crystals were obtained by addition of light petroleum, but the equiv. of the product was too low, as this was contaminated with fumaric acid. When larger quantities of the crude product were analogously recrystallised the product was not pure either.

That the product obtained was really cyclohexylsulphinylsuccinic acid was proved by oxidising it to cyclohexylsulphonylsuccinic acid. A solution of 1.7 g of a product, that had been stored for one year, in 20 ml of glacial acetic acid was treated with 2 ml of 30 % hydrogen peroxide. The next day the mixture was evaporated to dryness on a water bath. The crystalline cake obtained was dissolved in a few ml of water. Cooling on ice precipitated some crystal powder (fumaric acid), that was sucked off. The filtrate was concentrated and seeded with cyclohexylsulphonylsuccinic acid. The yield was 0.4 g of large, clear crystals of m.p. 78–80° and equiv. 140.8. Cyclohexylsulphonylsuccinic acid (p. 208) has m.p. 81–83° and equiv. 141.2.

At room temperature a solution of sodium cyclohexylsulphinylsuccinate was rather stable. A neutral solution prepared from 0.5 g of the acid, 4 ml of water, and 4.1 ml of N-NaOH was left overnight, then treated with 4 ml of N-HCl and evaporated to dryness over phosphorus pentoxide. From the residue 0.5 g of cyclohexylsulphinylsuccinic acid was extracted with acetone. The equiv. was 129.4 and the m.p. as above.

Owing to the difficulty of preparing large amounts of pure cyclohexylsulphinylsuccinic acid the following experiments were carried out with solutions of the sodium salt of the acid, prepared by oxidation of cyclohexylthiosuccinate with the calculated amount of hydrogen peroxide. This oxidation proceeds quantitatively, rather rapid, and without formation of more oxidised products. A neutral solution of cyclohexylthiosuccinate was prepared by dissolving 4.64 g of cyclohexylthiosuccinic acid in 40.00 ml of N-NaOH. The solution was cooled in water and oxidised with 4.47 ml of 4.47 M-$H_2O_2$. The volume was made up to 50.00 ml, after $T$ hours 2.00 ml diluted with water, acidified with sulphuric acid, treated with potassium iodide and a few drops of aqueous ammonium molybdate, and titrated with standard thiosulphate. The hydrogen peroxide not consumed was $p$ %, Table 2.

*Table 2*

| $T$ | 3 | 21 | 46 |
|---|---|---|---|
| $p$ | 15.8 | 1.4 | 0.6 |

Five ml of the oxidised solution were then treated with 0.2 ml of 10 M-$H_2O_2$ and the hydrogen peroxide not consumed was determined as above on 1 ml samples, Table 3.

*Table 3*

| $T$ | 0 | 3 | 24 | 72 |
|---|---|---|---|---|
| $p$ | 100.0 | 99.5 | 96.3 | 91.3 |

**Decomposition in alkaline solution.** Half of the above-mentioned solution of cyclohexylsulphinylsuccinate was treated with 10.00 ml of N-NaOH and heated in a water bath for 4 hours. When cooled down it was neutralised with 6.80 ml of N-HCl, and the oily solution extracted with ether. Evaporation and drying at the pump on a water bath afforded 0.66 g of dicyclohexyl disulphide of $n_D^{20}$ 1.5470, for the pure compound 1.5454 (1).

Acidification of the aqueous solution with 8 ml of 5 N-HCl precipitated 0.8 g of fumaric acid, after recrystallisation from water of equiv. 59.6. The mother-liquor was extracted three times with 40 ml of ether and the ethereal solutions neutralised with 5.50 + 1.50 + 0.90 = 7.90 ml of N-NaOH. The neutral, aqueous solution with a solution of 1.5 g of mercuric chloride in 10 ml of warm water yielded 0.8 g of a crystalline precipitate of chloromercury cyclohexylsulphinate containing 52.21 % Hg, calc. 52.34 %.

**Decomposition in neutral solution.** A neutral solution of 20 millimoles of cyclohexylsulphinylsuccinate, prepared as above, was heated on a water bath for 6 hours. It was then still neutral, and a faint odour of cyclohexanone was noticed. Some oil was taken up in ether, this evaporated and the residue dried at the pump on a water bath, affording 0.56 g of an oil of $n_D^{20}$ 1.5696, and according to the analysis consisting of a mixture of dicyclohexyl disulphide with sulphur or dicyclohexyl trisulphide. An authentic solution of the same amount of sulphur in dicyclohexyl disulphide had $n_D^{20}$ 1.5636, but was oversaturated at room temperature, whereas the former product on seeding with sulphur, gave no precipitation thereof.

For $(C_6H_{11})_2S_2 + 0.664\ S = C_{12}H_{22}S_{2.664}$ (251.71):

calc.: C 57.26 H 8.81 S 33.93 %
found: » 57.02 » 8.75 » 33.78 »

In another experiment, carried out as above, 20 ml of the turbid solution were distilled off after the heating. The distillate was filtered, removing a minute amount of oil, and the clear filtrate added to a hot solution of 0.4 g of 2,4-dinitrophenylhydrazine in 60 ml of 3 N-HCl. There was formed 0.45 g of a hydrazone of m.p. 159–161°, after recrystallisation from ethyl acetate-methanol, m.p. 160–162°, undepressed in admixture with authentic 2,4-dinitrophenylhydrazone of cyclohexanone. ALLEN gives the m. p. 160° (37).

The extracted aqueous solution from the first experiment was made acid with 10.00 ml of 5.00 N-HCl. Carbon dioxide was evolved and an emulsion formed, which rapidly gave a cristalline precipitate weighing 1.00 g, m.p. 82–84°. It was very soluble in the common organic solvents, excepting light petroleum, but recrystallisation from this did not improve the purity of the compound, which was rather unstable. It consisted of 1-cyclohexylthioacrylic acid, the properties of which will be further described in another paper (13).

For $CH_2{=}C(SC_6H_{11})COOH = C_9H_{14}O_2S$ (186.26):

calc.: C 58.03 H 7.58 S 17.21 % equiv. 186.3
found: » 58.44 » 7.60 » 16.89 » » 185.6

The mother-liquor was freed from carbon dioxide by evacuation, neutralised with 39.46 ml of N-NaOH, and made up to 150 ml. In one third of this solution the amount of cyclohexylsulphinylsuccinic acid not decomposed was determined by heating with 6.98 ml of N-NaOH in the way described above. After 4 hours in a water bath the solution was neutralised with 3.16 ml of 2 N-HCl and 0.14 g of dicyclohexyl disulphide isolated. A minute amount of cyclohexanesulphinic acid was extracted from the acidified aqueous solution with benzene and then fumaric acid, corresponding to 8.37 ml of N-NaOH, with ether by means of an efficient, continuous extractor. In the other two thirds of the solution cyclohexanesulphinic acid was sought for, but not found.

**Decomposition in acid solution.** To a solution of 40.00 millimoles of cyclohexylsulphinylsuccinate there were added 6.00 g of mercuric chloride and 15.30 ml of 5.23 N-$H_2SO_4$ and the mixture heated on a water bath for 2 hours. A slow stream of carbon dioxide-free air was aspirated through the reaction mixture, dried with magnesium perchlorate, and the carbon dioxide formed, absorbed in a weighed tube with sodium hydroxide-asbestos. The gain in weight was 0.8345 g. In the reaction mixture was formed a mass of small, white, crystal flakes, which were sucked off, washed with water, and then with acetone, until nothing more was dissolved. The remainder, 6.304 g, consisted mainly of chloromercury cyclohexyl mercaptide, contaminated with some mercury cyclohexyl mercaptide and calomel (grey with ammonia).

For $0.778\ C_6H_{11}SHgCl + 0.111\ (C_6H_{11}S)_2Hg + 0.267\ HgCl$ (384.18):

calc.: Cl 9.65 S 8.35 Hg 60.36 %
found: » 9.58 » 8.36 » 59.99 »

The acetone washings were diluted with ether and neutralised with 28.22 ml of N-NaOH. Evaporation of the acetone-ether and drying of the residue at the pump on a water bath gave 0.846 g of an oil containing 24.51 % S, which largely crystallised overnight. The crystals were drained on porous tile and identified as the cyclohexyl ester of cyclohexanethiolsulphonic acid, m.p. 37–40° (described on p. 193). The neutral aqueous solution was diluted to 250 ml and acidified with 6.00 ml of 5.02 N-HCl and gave an emulsion, overnight solidifying to 1.878 g of crystals of m.p. 95–112° and equiv. 311.3. Digestion with 100 ml of light petroleum (b.p. 80–100°) dissolved unidentifiable acid products corresponding to 0.85 ml of N-NaOH and digestion with 50 ml of water then dissolved acid material corresponding to 0.30 ml of N-NaOH. The equiv. of the undissolved product rose to 330.6, and it was finally purified by recrystallisation from ethyl acetate-light petroleum (b.p. 80–100°) to yield pure 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid of m.p. 116–118°, equiv. 334.1, calc. 334.5. This compound will be described by the author in another connection (13). The mother-liquor of the crude product was freed from some acetone by slight evaporation at the pump, a further small amount of impure 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid (corresp. to 0.25 ml of N-NaOH) filtered off, the filtrate neutralised with 23.80 ml of N-NaOH, and evaporated at about 30° at the pump to a volume of 25 ml. Acidification with 5.00 ml of 5.02 N-HCl afforded 1.141 g of impure fumaric acid, equiv. 68.1. Recrystallisation from ethyl acetate gave pure fumaric acid, equiv. 58.0, but no other product could be isolated. The mother liquor of the crude product was exhaustively extracted with ether in an efficient, continuous extractor. The extracted solution was neutralised with 3.40 ml of N-NaOH, and the largely crystalline extract digested twice with 50 ml of benzene. Acid material corresponding to 1.45 + 0.15 = 1.60 ml of N-NaOH was dissolved and the neutral, aqueous solution taken together with the corresponding one obtained later. The undissolved extract consisted of fumaric acid corresponding to 3.30 ml of N-NaOH, equiv. 59.1.

The aqueous mother-liquor and washings from the cyclohexyl mercaptide precipitate were made up to 200.0 ml, 5.00 ml thereof after addition of potassium bromide neutralised with 12.80 ml of 0.1003 N-NaOH, and added to a hot solution of 0.1 g of 2,4-dinitrophenylhydrazine in 10 ml of 5 N-HCl. There was formed 0.0569 g of crude 2,4-dinitrophenylhydrazone of pyruvic acid of m.p. 191–200°, recrystallised from methanol, m.p. 215–216°. SIMON and NEUBERG (45) report the m.p. 216°. The remainder of the solution was treated with 50 ml of N-NaOH, 1.6004 g of potassium xanthate, and then immediately exactly neutralised, the consumption of N-NaOH making a total of 50.70 ml. The next day the precipitate of mercury xanthate was filtered off, the filtrate acidified with 2.87 ml of 5.23 N-$H_2SO_4$, and after 2 hours neutralised with 10.80 ml of N-NaOH. The solution was evaporated at the pump at about 30° to a volume of 30 ml. Acidification with 7.65 ml of 5.23 N-$H_2SO_4$ precipitated 0.866 g of impure fumaric acid, equiv. 63.5, after recrystallisation from ethyl acetate, 58.5. From the turbid mother-liquor light petroleum extracted 0.047 g of dicyclohexyl disulphide of $n_D^{20}$ 1.5433. The clear solution was then made up to 60.0 ml, 5.00 ml thereof added to a hot solution of 0.2 g of 2,4-dinitrophenylhydrazine in 15 ml of 5 N-HCl, to give 0.1910 g of crude hydrazone, recrystallised from methanol and identified as the 2,4-dinitrophenylhydrazone of pyruvic acid, m. p. and mixed m. p. with authentic material 216–217° (decomp.), N-content 20.75 %, calc. 20.89 %. The remainder of the solution was then exhaustively extracted with ether in an extractor. The extracted solution was neutralised with 7.63 ml of N-NaOH and made up to 100.0 ml. Five ml were titrated according to Volhard, and contained chloride ions corresponding to 10.94 ml of 0.1 N-$AgNO_3$. The remainder almost immediately gave a persistent colour with potassium permanganate. The extract, after evaporation of the ether, consisted of a sticky crystalline mass, which was digested 3 times with 50 ml of warm benzene. The undissolved residue consisted of fumaric acid corresponding to 6.75 ml of N-NaOH, equiv. 58.9. The benzene solutions were neutralised with 7.70 + 1.10 + 0.20 = 9.00 ml of N-NaOH and the neutral aqueous solution, combined with the one obtained previously under similar conditions, treated with a solution of 0.6 g of S-benzyl-*iso*-thiuroniumchloride in 20 ml of water. A precipitate was formed weighing 0.3 g and of m.p. 130–140°. The turbid solution in 3 ml of methanol was filtered and precipitated with 15 ml of acetone, to yield 0.09 g of crystal crust of m.p. 146–148°. According to the analysis it consisted of the neutral S-benzyl-*iso*-thiuroniumsalt of a dibasic acid, the elementary composition of which corresponded to an addition product of 1-cyclohexylthioacrylic acid and pyruvic acid. The low yield of the compound made a closer investigation of its nature impossible.

For $(C_6H_5CH_2S \cdot C(NH_2):NH)_2$, $CH_2 = C(SC_6H_{11})COOH$, $CH_3COCOOH =$ $C_{28}H_{38}O_5N_4S_3$ (606.80):

| | | | | |
|---|---|---|---|---|
| calc.: | C 55.42 | H 6.31 | N 9.24 | S 15.85 % |
| found: | » 55.26 | » 6.18 | » 9.19 | » 15.79 » |

Pyruvic acid does not give a water-insoluble *iso*-thiuronium salt.

The results may be summarised thus. On heating, an aqueous solution of 1 mole of cyclohexylsulphinylsuccinic acid, in the presence of mercuric chloride, yielded (isolated as crude products or calculated from the titrations) 0.41 mole of chloromercury cyclohexyl mercaptide, 0.087 mole of cyclohexyl cyclohexanethiolsulphonate, probably contaminated with dicyclohexyl disulphide, 0.16 mole of 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid, 0.04 mole of an acid, possibly constituting an addition product between 1-cyclohexylthioacrylic acid and pyruvic acid, 0.22 mole of pyruvic acid, 0.54 mole of fumaric acid, and 0.47 mole of carbon dioxide. These results account for 94 % of the cyclohexylthio-group and 100 % of the succinic acid carbon skeleton.

Experiments were also carried out, in much the same way, with excess of sulphuric acid and with the acid originally half neutralised. In both these cases some of the products were less pure so the quantitative result was less reliable, but on the whole the same products were formed. In the acid solution there were formed larger amounts of cyclohexanethiol, the dubious addition compound, pyruvic acid, and carbon dioxide, and less of the other compounds. In the half neutralised solution the case was reversed with the exception, that the amount of 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid was less in this case too.

Without mercuric chloride, or with hydrochloric acid instead of sulphuric acid, the result was very different. Excess of chloride ions made the mercaptide sufficiently soluble to allow it to react, as did the free thiol, with the cyclohexane-sulphenic and -sulphinic acids, formed as intermediates, yielding dicyclohexyl disulphide. The formation of 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid was then also inhibited, and the intermediate 1-cyclohexylthioacrylic acid decomposed to cyclohexanethiol and pyruvic acid (see 13). The only products identified were thus cyclohexanethiol, dicyclohexyl disulphide, fumaric and pyruvic acid, and carbon dioxide. Besides, small amounts of unidentified, oily material was formed, especially in the absence of mercuric chloride (compare p. 194).

## IV. Cyclohexyl sulphinimines

### 1. Indifferent cyclohexyl sulphinimines

### a. Cyclohexylmethylsulphine-benzenesulphonylimine

A solution of 1.3 g of cyclohexyl methyl sulphide in 30 ml of acetone was treated with a solution of 2.6 g of chloramine-B[1] in 20 ml of water. The emulsion formed soon got clear and some heat was emitted. Free evaporation of the acetone separated an oily bottom layer, which solidified when seeded with a crystalline specimen, prepared by scratching a sample of the oil on a watch glass. The product melted after preliminary softening at 73–79°. Recrystallisation from xylene yielded small needles of m.p. 80.5–82.5°.

For $C_6H_{11} \cdot S(CH_3){:}NSO_2C_6H_5 = C_{13}H_{19}O_2NS_2$ (285.41):

calc.: C 54.70 H 6.71 N 4.91 S 22.47 %
found: » 54.69 » 6.76 » 4.98 » 22.47 »

### b. Dicyclohexylsulphine-benzenesulphonylimine

The reaction between 1.0 g of dicyclohexyl sulphide in 10 ml of acetone and 1.3 g of chloromine-B in 10 ml of water proceeded just as the foregoing one. The yield of crude product was 1.7 g of rod-shaped crystals of m.p. (after preliminary softening) 106–110°. Recrystallisation from xylene yielded 1.3 g of rod-crystals of m.p. 107.5–110.5°.

For $(C_6H_{11})_2S{:}NSO_2C_6H_5 = C_{18}H_{27}O_2NS_2$ (353.53):

calc.: C 61.15 H 7.70 N 3.96 S 18.14 %
found: » 61.45 » 7.72 » 4.10 » 18.18 »

### c. Cyclohexylphenylsulphine-benzenesulphonylimine

As above 1.0 g of cyclohexyl phenyl sulphide and 1.3 g of chloramine-B yielded 1.7 g of a crude product of m.p. 105–110°. Recrystallisation from ethanol gave aggregates of small rods of m.p. 113–114.5°.

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[1] The formula of this compound is reported as $C_6H_5SO_2NClNa$, 3 $H_2O$ (46). However, the author found, that the composition of material recrystallised from water and dried in the air corresponded to the formula $C_6H_5SO_2NClNa$, $1\frac{1}{2}$ $H_2O$.

For $C_6H_{11} \cdot S(C_6H_5):NSO_2C_6H_5 = C_{18}H_{21}O_2NS_2$ (347.48):

| | | | | |
|---|---|---|---|---|
| calc.: | C 62.21 | H 6.10 | N 4.03 | S 18.45 % |
| found: | » 62.37 | » 6.08 | » 4.03 | » 18.45 » |

### d. Cyclohexylbenzylsulphine-benzenesulphonylimine

From 1.0 g of cyclohexyl benzyl sulphide and 1.3 g of chloramine-B the yield of crude material was 1.8 g, m.p. 140–142°. It was recrystallised from 10 ml of ethanol to yield 1.4 g of small crystal rods of m.p. 142.5–143.5°.

For $C_6H_{11} \cdot S(CH_2C_6H_5):NSO_2C_6H_5 = C_{19}H_{23}O_2NS_2$ (361.50):

| | | | | |
|---|---|---|---|---|
| calc.: | C 63.12 | H 6.41 | N 3.88 | S 17.74 % |
| found: | » 63.19 | » 6.46 | » 4.00 | » 17.70 » |

## 2. The reactions between salts of cyclohexylthio-carboxylic acids and chloramine-B

### a. Sodium cyclohexylthioacetate and chloramine-B

A neutral solution was prepared from 1.74 g of cyclohexylthioacetic acid and 10.13 ml of N-NaOH. A solution of 2.5 g of chloramine-B in 10 ml of lukewarm water was cooled and added drop by drop. During the reaction the mixture was cooled with ice + salt. It first became turbid and then small, white crystals separated. After one hour the mixture was extracted 3 times with 25 ml of ethyl acetate. Free evaporation of the extract afforded 0.45 g of somewhat impure benzenesulphonamide of m.p. 147–149°, after recrystallisation from water, m.p. 152–153.5°. The m.p. reported in the literature is e. g. 153° (47). Acidification of the extracted, neutral solution with 5.10 ml of 2.00 N-HCl precipitated a very viscous oil, which was impossible to bring into crystalline form. It was filtered off. The filtrate was neutralised with 3.50 ml of N-NaOH. The oil, which was insoluble in ether, was taken up in ethyl acetate. The solution was washed with water, the solvent evaporated at the water pump at room temperature, the residue washed twice with water and twice with ether and finally dried at the water pump at room temperature. According to analysis it consisted of impure cyclohexylcarboxymethylsulphine-benzenesulphonylimine. The substance was unstable. Left in the air for a day it began to smell of cyclohexanethiol and became turbid from small crystals, but it did not crystallise altogether. Probably it was decomposed with formation of benzenesulphonamide.

For $C_6H_{11} \cdot S(CH_2COOH):NSO_2C_6H_5 = C_{14}H_{19}O_4NS_2$ (329.42):

| | | | | | |
|---|---|---|---|---|---|
| calc.: | C 51.04 | H 5.81 | N 4.25 | S 19.46 % | equiv. 329.4 |
| found: | » 50.46 | » 6.01 | » 4.04 | » 17.86 » | » 364 |

The neutralised filtrate from the oil was acidified with 5 ml of 2 N-HCl and then extracted with 50 ml of ether, which removed acid material corresponding to 0.90 ml of N-NaOH. No crystalline product was obtained from it. The extracted solution was treated with 1 g of mercuric chloride in the way described relevant to cyclohexylsulphinylacetic acid, p. 195, and yielded 0.65 g of chloromercury cyclohexyl mercaptide of equiv. 352.3, and then 0.3 g of crude glyoxylic acid phenylhydrazone of m.p. 127–130° (decomp.), equiv. 169.9.

Thus the chloramine to about 70 % reacted with sodium cyclohexylthioacetate to give the corresponding sulphinimine. In a parallel reaction the chloramine oxidised the sulphide to the corresponding sulphoxide.

### b. Sodium 2-cyclohexylthiopropionate and chloramine-B

A neutral solution of 1.88 g of 2-cyclohexylthiopropionic acid in 10.17 ml of N-NaOH was treated with a solution of 2.5 g of chloramine-B in 10 ml of water in the way previously described. Almost immediately light crystals began to separate. After one hour the mixture was extracted as above to yield 1.30 g of benzenesulphonamide of m. p. 148–150°. Acidification of the neutral solution with 5.10 ml of 2.00 N-HCl precipitated a very viscous oil, that did not crystallise. It was filtered off, and the filtrate neutralised with 7.70 ml of N-NaOH. The oil was treated as in the foregoing experiment and yielded impure cyclohexyl-2-carboxyethylsulphine-benzenesulphonylimine. When left in the air the substance soon smelt of acrylic acid.

For $C_6H_{11} \cdot S(CH_2CH_2COOH){:}NSO_2C_6H_5 = C_{15}H_{21}O_4NS_2$ (343.45):

| | | | | | |
|---|---|---|---|---|---|
| calc.: | C 52.45 | H 6.16 | N 4.08 | S 18.67 % | equiv. 343.5 |
| found: | » 52.19 | » 6.77 | » 3.42 | » 17.71 » | » 350 |

The neutralised filtrate from the sulphinimine was acidified with 5 ml of 2 N-HCl and extracted with 50 ml of ether, which removed acid material corresponding to 1.20 ml of N-NaOH. No crystalline material was obtained from it. Ethyl acetate then extracted acid corresponding to 3.65 + 1.85 + 1.15 + + 0.70 = 7.35 ml of N-NaOH. From the aqueous solution of the extract, acidification, extraction with ethyl acetate, and free evaporation of this solvent yielded 1.2 g of material of m.p. 85–100° and equiv. 231.4. Recrystallisation from ethyl acetate gave somewhat impure 2-cyclohexylsulphinylpropionic acid of m.p. 100–110° and equiv. 207.2. Further recrystallisations from ethyl acetate and water did not improve the purity.

In this case the reaction yielded only about 25 % sulphinimine and the chloramine oxidised the rest mainly to the sulphoxide.

### c. Sodium cyclohexylthiosuccinate and chloramine-B

The reaction was carried out as in the foregoing experiments. From 2.32 g of cyclohexylthiosuccinic acid, 20.00 ml of N-NaOH, and 2.5 g of chloramine-B in 10 ml of water a porridge of small crystals of benzenesulphonamide was obtained. The yield was 1.55 g, m.p. 148–150°. In one experiment the neutral, extracted solution was acidified and separated an infinitesimal amount of an oil. The cyclohexylsulphinylsuccinic acid supposed to be formed could not be isolated on account of its instability. In another experiment the extracted, neutral solution was therefore heated in a water bath for 10 hours. The same products resulted as in the direct experiment (p. 198) with sodium cyclohexylsulphinylsuccinate, namely 0.45 g of sulphur-containing dicyclohexyl disulphide of $n_D^{20} = 1.5660$, 0.65 g of 1-cyclohexylthioacrylic acid of m.p. 77–80° and equiv. 189.8, and 0.85 g of crude fumaric acid, recrystallised from water, of equiv. 60.2. In this case the chloramine thus reacted exclusively or almost exclusively with formation of sulphoxide.

## V. Cyclohexyl sulphones

### 1. Indifferent cyclohexyl sulphones

### a. Cyclohexyl methyl sulphone

A solution of 3.9 g of cyclohexy -methyl sulphide in 50 ml of glacial acetic acid was oxidised with 10 ml of 30 % hydrogen peroxide. The temperature rose to about 60°. After some hours the acetic acid was distilled off at the water pump, the metal bath finally reaching 150°. The residue, 4.3 g, was a brown oil, which was fractionated at a pressure of 0.8 mm. The main portion, 2.4 g of a colourless oil, boiled at 118–119°. Cooled in ice and scratched, it solidified and melted again at 19–23°. For cyclohexyl methyl sulphone BARNARD, FABIAN, and KOCH (39) report b.p. 94.5–95°/0.05 mm, $n_D^{20}$ 1.4918.

For $C_6H_{11} \cdot SO_2 \cdot CH_3 = C_7H_{14}O_2S$ (162.24):

| | | | |
|---|---|---|---|
| calc.: | C 51.82 | H 8.70 | S 19.76 % |
| found: | » 51.50 | » 8.73 | » 19.76 » |

$d_4^{20}$ 1.1455, $n_D^{20}$ 1.4900, mol.refr. 40.95, refr. of the sulphonyl-group 8.62.

For the refraction of the sulphonyl-group STRECKER and SPITALER (41) report 8.61.

### b. Dicyclohexyl sulphone

A solution of 2.0 g of dicyclohexyl sulphide in 20 ml of glacial acetic acid was treated with 4 ml of 30 % hydrogen peroxide. On shaking, a clear solution resulted and the temperature rose to about 40°. Free evaporation afforded 2.3 g of large, rod-shaped crystals of m.p. 129–132°. Recrystallisation from 5 ml of ethanol yielded 1.9 g of rod crystals of m.p. 133.5–135°. BORSCHE and LANGE (48) report the m.p. 132–132.5.

For $(C_6H_{11})_2SO_2 = C_{12}H_{22}O_2S$ (230.36):

| | | | |
|---|---|---|---|
| calc.: | C 62.56 | H 9.63 | S 13.92 % |
| found: | » 62.67 | » 9.52 | » 13.87 » |

### c. Cyclohexyl phenyl sulphone

This compound was prepared analogously, but the acetic acid solution was heated for one hour and a half on a water bath. Otherwise the oxidation was not complete. From 1.9 g of cyclohexyl phenyl sulphide there was obtained

2.25 g of crude product of m.p. 72–74.5°. Recrystallisation from 5 ml of glacial acetic acid yielded 0.4 g of transparent, almost quadratic tablets of m.p. 73–76°. GILMAN and HECK (49) give the m.p. 73°, and CUNNEEN (50) reports the m.p. 74°.

For $C_6H_{11} \cdot SO_2 \cdot C_6H_5 = C_{12}H_{16}O_2S$ (224.31):

| | | | |
|---|---|---|---|
| calc.: | C 64.25 | H 7.19 | S 14.29 % |
| found: | » 64.65 | » 7.13 | » 14.28 » |

### d. Cyclohexyl benzyl sulphone

From 2.1 g of cyclohexyl benzyl sulphide there was obtained as above 2.4 g of a crude product of m.p. 96–100°. Recrystallisation from 5 ml of ethanol yielded 1.85 g of glistening crystal flakes of m.p. 104–104.5°.

For $C_6H_{11} \cdot SO_2 \cdot CH_2C_6H_5 = C_{13}H_{18}O_2S$ (238.33):

| | | | |
|---|---|---|---|
| calc.: | C 65.51 | H 7.61 | S 13.45 % |
| found: | » 65.66 | » 7.54 | » 13.50 » |

An attempt was made to add toluene-ω-sulphinic acid to cyclohexene in ethereal solution, but no reaction was observed.

## 2. Cyclohexylsulphonyl-carboxylic acids

### a. Cyclohexylsulphonylacetic acid

A solution of 1.75 g of cyclohexylthioacetic acid in 20 ml of glacial acetic acid was cooled in ice and then treated with 4 ml of 30 % hydrogen peroxide. The reaction mixture was kept on ice for four hours and then left at room temperature overnight. Evaporation on a water bath gave a residue, that crystallised to a radiant mass weighing 1.9 g. It melted at 60–65° and had the equiv. 209.5. After two recrystallisations from 3 ml of benzene the m.p. rose to 82.5–84.5°. CUNNEEN (50) reports the m.p. 82°.

For $C_6H_{11} \cdot SO_2 \cdot CH_2COOH = C_8H_{14}O_4S$ (206.25):

| | | | | |
|---|---|---|---|---|
| calc.: | C 46.58 | H 6.84 | S 15.54 % | equiv. 206.3 |
| found: | » 46.50 | » 6.80 | » 15.48 » | » 207.0 |

On a larger scale it is preferable to use potassium permanganate as an oxidising agent. A neutral solution of sodium cyclohexylthioacetate was prepared from 8.7 g of cyclohexylthioacetic acid, 7.2 g of cryst. sodium carbonate, and 50 ml of water. In the course of 2.5 hours 270 ml of 0.75 N-$KMnO_4$ (calculated for reduction to $MnO_2$) were added by drops to the solution, through which carbon dioxide was bubbled. Only slight emission of heat was observed. The next day, the manganese dioxide was filtered off, the filtrate and washings evaporated to a volume of 35 ml, the solution being kept neutral with additions of 5 N-HCl. It was then acidified with 15 ml of 5 N-HCl to yield an emulsion and an oily bottom layer. Extraction with ether and evaporation gave 9.7 g of an oil, which crystallised to a radiant mass, m.p. 75–78°, equiv. 212.9. It was recrystallised from benzene-light petroleum, to yield 7.5 g of pure cyclohexylsulphonylacetic acid in the form of crystal flakes, m.p. 82.5–84.5°, equiv. 207.1.

The compound was stable when heated on a water bath in acid, neutral, or alkaline solution.

### b. 2-Cyclohexylsulphonylpropionic acid

This compound was prepared as the foregoing one.

From 1.9 g of cyclohexylthiopropionic acid and 4 ml of 30 % hydrogen peroxide in 20 ml of glacial acetic acid there was obtained 2.3 g of a crude product of m.p. 90–100°, equiv. 214.2. Recrystallisation from water gave a pure product, m.p. 111–113°, equiv. 219.8, calc. 220.3.

Following the other method a neutral solution of sodium 2-cyclohexylthiopropionate, prepared from 18.8 g of the acid, 14.3 g of cryst. sodium carbonate, and 100 ml of water, was oxidised with 550 ml of 0.72 N-$KMnO_4$. The filtrate from the manganese dioxide slime was acidified with 20 ml of conc. hydrochloric acid and evaporated on a water bath, by and by yielding an oil. When the volume was reduced to 100 ml, the mixture was cooled down and treated with a further 10 ml of conc. hydrochloric acid. The oil solidified to white crystal needles weighing 14.5 g, m.p. 110–113°, equiv. 221.8. From the filtrate 3.8 g of impure product was obtained by extraction with ether. Of the main portion 3.0 g were recrystallised from 10 ml of ethyl acetate yielding 1.9 g of white crystal needles of m.p. 112.5–114°.

For $C_6H_{11} \cdot SO_2 \cdot CH_2CH_2COOH = C_9H_{16}O_4S$ (220.28):

| | | | | |
|---|---|---|---|---|
| calc.: | C 49.07 | H 7.32 | S 14.55 % | equiv. 220.3 |
| found: | » 48.70 | » 7.12 | » 14.46 » | » 221.3 |

The compound was stable when heated on a water bath in acid or neutral, aqueous solution. In alkaline solution it was decomposed with formation of sodium cyclohexanesulphinate and acrylate.

A neutral solution of 1.1 g of 2-cyclohexylsulphonylpropionic acid in 5.00 ml of N-NaOH was made alkaline with a further 5.00 ml of N-NaOH. The solution was then heated on a water bath for 2.5 hours. It then got neutral with 0.35 ml of 2 N-$H_2SO_4$. Addition of a warm solution of 1.4 g of mercuric chloride in 8 ml of water gave a white porridge of microscopic crystal needles. When sucked off and dried the precipitate weighed 1.3 g. It began to get dark at 140°, but did not melt below 250°. It consisted of the chloromercury salt of cyclohexanesulphinic acid.

For $C_6H_{11} \cdot SO_2HgCl = C_6H_{11}O_2ClSHg$ (383.28):

| | | | |
|---|---|---|---|
| calc.: | Cl 9.25 | S 8.36 | Hg 52.34 % |
| found: | » 9.26 | » 8.33 | » 52.54 » |

The filtrate was acidified with 2.5 ml of 5 N-HCl and treated with 25 ml of 0.2 M toluene-ω-sulphinic acid solution. During 5 days 0.75 g of crystal flakes separated which, after recrystallisation from water, melted at 176–178° and had the equiv. 227.7, for 2-benzylsulphonylpropionic acid calc. 228.2.

### c. Cyclohexylsulponylsuccinic acid

In the preparation of this compound it is necessary to be careful, otherwise the product will be contaminated with fumaric acid, which is difficult to remove.

According to the first method a solution of 2.3 g of cyclohexylthiosuccinic acid in 25 ml of glacial acetic acid was oxidised with 4 ml of 30 % hydrogen peroxide, the mixture being cooled in ice overnight. After another day at room temperature the solution was evaporated on a water bath, until almost all acetic acid was removed. Some drops of water were added, and on being vigorously scratched the liquid solidified. The yield of air-dried material was 2.8 g, m.p. 75–110°, equiv. 142.0. It was recrystallised from 2 ml of water to a yield of 1.75 g of microscopic rods, m.p. 81–83° (open or sealed capillary).

For $C_6H_{11} \cdot SO_2 \cdot CH(COOH)CH_2COOH,\ H_2O = C_{10}H_{18}O_7S$ (282.30):

| | | | | | |
|---|---|---|---|---|---|
| calc.: | C 42.54 | H 6.43 | S 11.36 | $H_2O$ 6.38 % | equiv. 141.2 |
| found: | » 42.63 | » 6.41 | » 11.32 | » 6.48 » | » 141.6 |

According to the second method a neutral solution of sodium cyclohexylthiosuccinate, prepared from 11.6 g of the acid, 14.3 g of cryst. sodium carbonate, and 50 ml of water, was oxidised as before with 280 ml of 0.72 N-$KMnO_4$. The temperature of the solution was kept below 15° by occasional additions of ice. The filtrate from the manganese dioxide slime was acidified with 20 ml of conc. hydrochloric acid and then extracted with three 250 ml portions of ether. Evaporation afforded a residue which, after seeding with the above preparation, soon solidified to a crystalline cake, which was powdered and dried in the air. It weighed 13.0 g, melted at 75—79°, and had the equiv. 143.3. The crude product was thus almost pure.

The stability of cyclohexylsulphonylsuccinic acid was similar to that of 2-cyclohexylsulphonylpropionic acid. Thus no decomposition was observed when the compound was heated in aqueous solution of acidic or neutral reaction. In alkaline solution its salt was split into cyclohexanesulphinate and fumarate.

A neutral solution of 2.8 g of cyclohexylsuphonylsuccinic acid in 20.0 ml of N-NaOH was made alkaline with a further 10 ml of N-NaOH and then heated in a water bath. After half an hour it was neutralised with 3.52 ml of 0.992 N-HCl, then again made alkaline with 3.55 ml of N-NaOH, and heated as before. After a full two hours, the solution became neutral with 1.46 ml of 0.992 N-HCl, corresponding to 85 % decomposition. Acidification with 7 ml of 5 N-HCl precipitated 0.8 g of fumaric acid, equiv. 59.4, calc. 58.0. From the mother-liquor three 50 ml portions of ether extracted acids corresponding to 12.5 + 2.3 + 0.8 = 15.6 ml of N-NaOH. The neutral, ethereal solution gave no residue on evaporation. The neutral aqueous solution was treated with 8 ml of 2 N-$H_2SO_4$ and yielded an emulsion and some oil, which was dissolved after the addition of 10 ml of water. Addition of a warm solution of 2.7 g of mercuric chloride in 15 ml of water immediately precipitated 2.1 g of white crystal needles, consisting of the above-mentioned chloromercury salt of cyclohexanesulphinic acid, Hg-content 52.49 %, calc. 52.34 %.

# VI. Cyclohexanesulphinic acid

## 1. Preparation and characterisation

The formation of cyclohexanesulphinic acid by alkaline fission of 2-cyclohexylsulphonylpropionic or cyclohexylsulphonylsuccinic acid is already described. For preparative purposes it was found most suitable to use the latter acid and free the sulphinic acid altogether from the simultaneously formed fumaric acid. This was achieved in the following way.

A neutral solution of 14.1 g of cyclohexylsulphonylsuccinic acid in 100 ml of N-NaOH was treated with a further 60 ml of N-NaOH. The solution was heated in a water bath for 2 hours. It was then neutralised with 2.4 ml of 5 N-HCl. Acidification with 33 ml of 5 N-HCl afforded a crystal porridge, which was sucked off. Extraction of the crystals twice with 150 ml of benzene dissolved cyclohexanesulphinic acid corresponding to 21.6 + 1.7 ml of N-NaOH. The filtrate was likewise extracted 3 times with 150 ml of benzene and the extracts neutralised with 16.0 + 3.4 + 1.7 ml of N-NaOH. Thus a solution of sodium cyclohexanesulphinate corresponding to totally 44.4 ml of N-NaOH was obtained. As fumaric acid is practically insoluble in benzene the sulphinate was almost free from it. This solution was used in most of the following experiments.

The free cyclohexanesulphinic acid was rather unstable. Acidification of 12 ml of the above-mentioned solution with 3 ml of 5 N-HCl precipitated an oil, which on being cooled in ice and scratched, crystallised to 1.5 g of microscopic rods, which melted at 39–42° and had the equiv. 169.5. Recrystallisation from 35 ml of water afforded 0.35 g of long, glistening crystal rods of m.p. 38.5–41.5° (sealed capillary). It was immediately analysed. For a preparation of cyclohexanesulphinic acid, VON BRAUN and WEISSBACH (42) report the m.p. 33–35°. BORSCHE and LANGE (48) obtained the magnesium salt as an intermediate in the synthesis of cyclohexanethiol.

For $C_6H_{11} \cdot SO_2H,\ H_2O = C_6H_{14}O_3S$ (166.23):

| | | | | |
|---|---|---|---|---|
| calc.: | C 43.35 | H 8.49 | S 19.29 % | equiv. 166.2 |
| found: | » 43.56 | » 8.48 | » 19.23 » | » 167.1 |

Left in the air, cyclohexanesulphinic acid softened and melted within a few hours. In a vacuum desiccator over phosphorus pentoxide it softened immediately and rapidly lost in weight, but no sharp limit was found for this loss. A sample in a sealed tube did not deteriorate appreciably in a year.

The chloromercury salt of cyclohexanesulphinic acid previously described was soluble in aqueous sodium chloride. The acid therefore was not quantitatively precipitated with mercuric chloride, but almost so with a mixture of mercuric chloride and mercuric sulphate. When 5.0 ml of the cyclohexanesulphinate solution mentioned above (containing extractable acids corresp. to 4.5 ml of N-NaOH) was mixed with 10 ml of water and 2 ml of 5 N-$H_2SO_4$ and then treated with a solution of 1.35 g of mercuric chloride in 10 ml of warm water, the yield of microscopic tables was 1.0 g (calc. 1.7 g), Hg-content 52.79 %, calc. 52.34 %. In another experiment the same amount of sulphinate solution and water was treated with a solution of 0.68 g of mercuric chloride and

0.74 g of mercuric sulphate in 2 ml of 5 N-$H_2SO_4$ and 10 ml of water. A thick porridge of granules without distinct crystalline character resulted. The yield was 1.6 g (calc. 1.7 g), Hg-content 52.67 %.

When a solution of 1.0 g of S-benzyl-*iso*-thiuronium chloride in 20 ml of water was mixed with 5.0 ml of the above-mentioned cyclohexanesulphinate solution a thick, white porridge of badly formed, microscopic crystals resulted, yield 1.3 g, m.p. 154–156°. Recrystallisation from 45 ml of water afforded 1.1 g of large, thin, and clear tablets of m.p. 158–159°. A sample dried over phosphorus pentoxide had the same m.p., whereas a sample in a sealed capillary softened at 135° and gave a clear melt at 143°. According to the analysis the preparation had lost some of its water of crystallisation. The dried sample did not take up any water from the air, but the original preparation did not lose any water in the air during a day either.

For $C_6H_{11} \cdot SO_2H,\ H_2NC(:NH)SCH_2C_6H_5 + 0.889\ H_2O$:

calc.: C 50.88 H 7.25 N 8.48 S 19.40 $H_2O$ 4.85 %
found: » 50.98 » 7.17 » 8.48 » 19.40 » 4.85 » (Vac. $P_2O_5$)

With 2-naphthylamine hydrochloride the sulphinate gave a precipitate, but it was impossible to obtain a salt of stoichiometrical composition.

The stability of cyclohexanesulphinic acid was investigated under various conditions. Five ml of the mentioned cyclohexanesulphinate solution were left in a closed (but not filled) vessel for three months, when it was still neutral. After acidification, benzene extracted acids corresponding to 1.16 ml of N-NaOH. — When cyclohexanesulphinic acid was heated for 4 hours in aqueous solution, with or without sulphuric acid, or in neutral or alkaline solution no appreciable decomposition was observed. The acid could be extracted with benzene almost in quantity and precipitated as the chloromercury salt.

When the chloromercury salt was heated at 150–165° it was decomposed with a formation of cyclohexene (less than 20 % of the amount theoretically possible), sulphur dioxide (ca. 40 %), mercurous salts, and dark coloured, amorphous or tarry products. As the result was not a promising one, the experiments were not carried further.

### 2. Cyclohexanesulphinic acid and bromine

When 10 ml of a solution of sodium cyclohexanesulphinate, prepared as previously described, was treated with saturated bromine water, a white emulsion immediately resulted, which finally turned yellow. It was decolourised with sulphur dioxide water and then extracted with light petroleum. The solvent was evaporated and the residue dried at the pump on a water bath. Distillation of the remainder, 1.9 g, yielded 1.8 g of distillate of b.p. 95°/0.6 mm. In another experiment the sulphinate solution was acidified with 5 ml of 2 N-HCl and diluted with 20 ml of water before the treatment with bromine water. The result was altogether the same as before. When the bromide was cooled with ice + salt it solidified and melted again at 4–6°.

For $C_6H_{11} \cdot SO_2Br$ (227.13):

calc.: C 31.73 H 4.88 Br 35.19 S 14.12 %
found: » 31.83 » 4.80 » 35.02 » 14.11 »
$d_4^{20}$ 1.5439, $n_D^{20}$ 1.5333, mol.refr. 45.68, refr. of the $SO_2Br$ group 19.07.

### 3. Sodium cyclohexanesulphinate and chloramine-B

Twenty ml of sodium cyclohexanesulphinate solution (prepared as before) were treated with a solution of 4.86 g of chloramine-B (oxidation equiv. 121.5) in 20 ml of water, added in two portions. A precipitate of small, white flakes immediately was formed and the mixture became alkaline. It was neutralised (phenol red) with 2 N-$HNO_3$, 10.70 ml in all being consumed. After half an hour a drop of the mixture still oxidised aqueous potassium iodide, so two 2 ml portions of sulphinate solution were added, each time neutralised, with 1.40 and 1.15 ml of 2 N-$HNO_3$. The chloramine was then consumed, as the mixture did not oxidise aqueous potassium iodide. The mixture was shaken with light petroleum, the undissolved matter filtered with suction, and washed with water and light petroleum. It consisted of 2.05 g of white crystal flakes of benzenesulphonamide of m.p. 152–153°.

The petroleum layer was evaporated, the residue dried at the pump on a water bath (residue 2.34 g), and then distilled to yield 2.30 g of cyclohexanesulphonyl chloride of b.p. 79°/0.9 mm.

For $C_6H_{11} \cdot SO_2Cl$ (182.67):

| | | | | |
|---|---|---|---|---|
| calc.: | C 39.45 | H 6.07 | Cl 19.41 | S 17.55 % |
| found: | » 39.60 | » 6.07 | » 19.40 | » 17.54 » |

$d_4^{20}$ 1.2696, $n_D^{20}$ 1.4977, mol.refr. 42.15, refr. of the $SO_2Cl$ group 15.54.

Borsche and Lange (48) for the same compound report b.p. 127–128°/15 mm, and Sprague and Johnson (51) b.p. 122–123°/13 mm, $n_D^{25}$ 1.4960. Kolker and Lapworth (52) give the m.p. 106°, which seems dubious. Landau (53) gives the value 15.40 for the refraction of the sulphonyl chloride group, and Hedlund (54, p. 19) the value 15.36.

The aqueous layer was made up to 150 ml. Twentyfive ml were titrated according to Volhard. The chloride content corresponded to 11.60 ml of 0.1000 N-$AgNO_3$, whereas the amount calculated from the quantity of chloramine-B used and cyclohexanesulphonyl chloride formed was 11.98 ml. Ethyl acetate from the remainder of the aqueous layer extracted 0.64 g of crude benzenesulphonamide.

The extracted solution was acidified with 5 ml of 5 N-HCl. A transient turbidity was observed. The solution was extracted three times with 50 ml of ether, which was neutralised with $1.95 + 0.55 + 0.30 = 2.80$ ml of N-NaOH. Acidification of the neutral extract solution precipitated an oil, which on being scratched crystallised to 0.43 g of a product of m.p. 168–172°. Recrystallisation from benzene afforded 0.35 g of glistening, soft needles, m.p. 170–172°. According to analysis it consisted of cyclohexanesulphonylbenzenesulphonamide. Similar derivatives of methanesulphonylamide (55) and sulphanilamide (56) were lately described.

For $C_6H_{11} \cdot SO_2 \cdot NH \cdot SO_2 \cdot C_6H_5 = C_{12}H_{17}O_4NS_2$ (303.39):

| | | | | | | |
|---|---|---|---|---|---|---|
| calc.: | C 47.50 | H 5.65 | N 4.62 | S 21.13 % | equiv. | 303.4 |
| found: | » 47.76 | » 5.83 | » 4.67 | » 21.02 » | » | 306 (phenolphthalein) |

This compound remained unchanged when heated for 4 hours in a water bath in acid or in alkaline solution. It could not be prepared by the action of cyclohexanesulphonyl bromide or chloride on benzenesulphonamide in pyridine. The sulphonyl halogenide was decomposed and the reaction not further investigated.

The aqueous solution, from which the cyclohexanesulphonylbenzenesulphonamide had been extracted, was evaporated to dryness in vacuo on a water bath, the residue dissolved in 10 ml of water, and neutralized with a little N-NaOH. It was then evaporated to dryness again, the remainder extracted with ethyl acetate (practically nothing dissolved), and then twice extracted with 50 ml of boiling absolute ethanol. On cooling, the first portion yielded 0.24 g of glistening crystal flakes. The mother-liquor and the second portion were evaporated and the residue recrystallised from 45 ml of absolute ethanol to yield 0.45 g of crystal flakes. Recrystallisation of the joined products from a few ml of water finally yielded pure sodium cyclohexanesulphonate, which in a sealed capillary softened from 190° to an opaque mass, but did not give a clear melt below 220°. The same compound was described by KOLKER and LAPWORTH (52).

For $C_6H_{11} \cdot SO_3Na, H_2O = C_6H_{13}O_4SNa$ (204.22):

calc.: C 35.29 H 6.41 S 15.70 Na 11.26 $H_2O$ 8.82 %
found: » 35.29 » 6.46 » 15.69 » 11.25 » 8.82 »

Counted on 1 mole of cyclohexanesulphinate and 1 mole of chloramine-B the following amounts were isolated (calc. from the crude products): 0.90 mole of benzenesulphonamide, 0.64 mole of cyclohexanesulphonyl chloride, 0.09 mole of cyclohexanesulphonylbenzenesulphonamide, and 0.21 mole of sodium cyclohexanesulphonate. These quantities account for 94 % of the $C_6H_{11}S$-radical and 99 % of the $C_6H_5SO_2N$-radical. The amount of nitric acid consumed was 1.33 mole, calc. from the reaction products 1.28 mole.

### 4. Cyclohexanesulphinic acid and unsaturated compounds

#### a. Cyclohexanesulphinic acid and acrylic acid

A neutral solution of 8.81 g of 2-cyclohexylsulphonylpropionic acid in 39.80 ml of N-NaOH was made alkaline, 82,88 ml of N-NaOH being totally used. The solution was heated for 2.5 hours in a water bath. After being cooled it was neutralised with 3.35 ml of 2 N-HCl, made up to 100.0 ml and divided into 4 equal parts, of which

the first was treated with 20 ml of water and 5 ml of 2 N acetic acid,
the second with 20 ml of water and 5 ml of 2 N-HCl
the third with 15 ml of water and 10 ml of 2 N-HCl
the fourth with 10 ml of water and 15 ml of 2 N-HCl.

The mixtures were kept for 20 hours at 20.5° and then the clear solutions were neutralised with 3.39, 2.17, 13.25, and 26.56 ml of N-NaOH. They were reacidified with 5 ml of 2 N-HCl each, and after 3 days put together and extracted with ether. The solvent was evaporated to yield 5.0 g of a crystal mass. Recrystallisation from water gave pure 2-cyclohexylsulphonylpropionic acid, m.p. 112–113.5°, equiv. 219.6.

Table 4 gives the calculated compositions and the amount of reaction in % (the small amount of NaCl formed in the neutralisation of the heated solutions is not considered). HA = acrylic acid, HS = cyclohexanesulphinic acid, HAc = acetic acid.

*Table 4*

| Composition | % Reaction |
|---|---|
| $C_{NaA} = C_{NaS} = C_{HAc} = 0.2$ | 69 |
| $C_{HA} = C_{NaS} = C_{NaCl} = 0.2$ | 82 |
| $C_{HA} = C_{HS} = \frac{1}{2} C_{NaCl} = 0.2$ | 71 |
| $C_{HA} = C_{HS} = C_{HCl} = \frac{1}{2} C_{NaCl} = 0.2$ | 36 |

### b. Cyclohexanesulphinic acid and maleic acid

A solution of 2.8 g of cyclohexylsulphonylsuccinic acid in 30 ml of N-NaOH was heated for 2 hours in a water bath. Acidification with 8 ml of 5 N-HCl precipitated 0.85 g of fumaric acid, which was filtered off. The filtrate was treated with a solution of 1.2 g of maleic acid in 5 ml of water. After 2 days the solution was extracted twice with 50 ml of ether, which was neutralised with 18.5 + 3.4 = 21.9 ml of N-NaOH. The neutral extract solution was acidified, extracted with ether, the ether evaporated, and the crystalline residue, 2.4 g, recrystallised from a few ml of water. Pure cyclohexylsulphonylsuccinic acid resulted, equiv. 140.9, m.p. 79–81°.

### c. Cyclohexanesulphinic acid and cyclohexene

An aqueous solution of cyclohexanesulphinic acid was extracted with cyclohexene to yield a solution of the sulphinic acid in that solvent. The reaction was followed by titrating samples of the mixture with standard alkali. Though the sulphinic acid was slowly consumed, no reaction product of interest could be isolated.

## VII. Reactions between chloramine-B and cyclohexanethiol, dicyclohexyl disulphide, cyclohexyl thiolactetate, cyclohexyl thiolbenzoate, and cyclohexyl cyclohexanethiolsulphonate

### 1. Cyclohexanethiol and chloramine-B

An alkaline solution of cyclohexanethiol was prepared as previously described (1) from 31 g of cyclohexyl thiolacetate and 24 g of caustic soda in a mixture of 100 ml of water and 15 ml of ethanol. A solution of 25 g of chloramine-B in 200 ml of water was added in small portions and the mixture shaken. A top layer separated and was taken up in light petroleum. Evaporation of the solvent left an oil, which was distilled at 1 mm to yield 18.2 g of dicyclohexyl disulphide of b.p. 130°, $n_D^{20}$ 1.5453. This was previously described (1). The aqueous layer was neutralised (phenol red) with 65 ml of 5 N-HCl. A thick porridge of crystals separated and a strong odour of cyclohexanethiol was noticed. The crystals were sucked off and washed with water and ether to yield 11.9 g of benzenesulphonamide of m.p. 153–155°. In another experiment chloramine-T was used. From 31 g of cyclohexyl thiolacetate and 30 g of chloramine-T the yields were 19 g of dicyclohexyl disulphide and 15.4 g of *p*-toluenesulphonamide of m.p. 136–138°. WOLKOW (57) reports the m.p. 137°.

## 2. Dicyclohexyl disulphide and chloramine-B

### a. Dicyclohexyl disulphide and chloramine-B in acetone-water

The disulphide was dissolved in acetone and aqueous chloramine-B added. In preliminary experiments it was observed, that even if only a very small amount of chloramine was added, the mixture oxidised aqueous potassium iodide for a considerable time. The pH of the mixture immediately after the addition fell from 7 to about 5. At pH 6 an aqueous solution of chloramine-B began to disproportionate, so it was concluded, that the dichloramine formed was responsible for the persistent oxidising properties of the reaction mixture. If this was neutralised (phenol red) by and by during the addition of chloramine, a rather well defined end point was reached when 4 moles of chloramine per mole of disulphide had been added.

A solution of 14.8 g of chloramine-B (oxidation equiv. 123.4) in 45 ml of water was added in portions to a cold solution of 3.46 g of dicyclohexyl disulphide in 75 ml of acetone. During the addition the mixture was cooled externally with water. The clear solution was then neutralised with N-NaOH (phenol red). A stable end point was reached when 26.50 ml had been added. A drop of the mixture did not oxidise aqueous potassium iodide. The acetone (and some water) was distilled off on a water bath. Two fractions were collected up to 76° in the vapour. The first one, 75 ml, contained chlorine-compounds corresponding to 0.61 ml of N-$AgNO_3$. There was also some indication of the presence of cyclohexene. The distillate was diluted with much water and extracted with ether. The ether was washed free from acetone with water, and then treated with 0.1 N bromine in glacial acetic acid until, after the addition of 20 ml, a yellow colour persisted. The solution was washed free from acetic acid with water and finally with N-NaOH. Free evaporation of the neutral ether left an infinitesimal amount of an oil, that had the typical odour of 1,2-dibromocyclohexane.

The second fraction, 3 ml of a turbid liquid, deposited a very small amount of glistening needles of m.p. 116—120°. Monoclinic sulphur melts at 119°. The crystals had all the properties of sulphur: they sublimed when heated, were soluble in benzene, carbon disulphide, and warm aqueous sodium sulphite. Warming with copper powder yielded a black product and burning in the air the odour of sulphur dioxide.

During the distillation the solution had turned acid and was neutralised with 2.35 ml of N-NaOH. The neutral solution was extracted with ether in an efficient, continuous extractor. Evaporation of the solvent left a sticky, crystalline residue (some of it was lost by an accident), which was extracted 3 times with 30 ml of light petroleum to leave 2.85 g undissolved. Recrystallisation from water yielded pure benzenesulphonamide of m.p. 153–154°.5. The petroleum extract was evaporated and yielded 0.4 g of an oil, from which no pure product was obtained. Most of it was insoluble in water and might have been unreacted disulphide.

The extracted, neutral, aqueous solution was made up to 150 ml. Five ml were diluted with 20 ml of water, acidified with 5 ml of 2 N-$HNO_3$, and extracted 3 times with 25 ml of chloroform. The extracted solution was titrated according to VOLHARD and found to contain chloride ion corresponding to 19.51 ml of 0.1 N-$AgNO_3$.

The main portion of the solution was then acidified with 10.00 ml of 5 N-HCl. The emulsion formed was extracted with the chloroform solution obtained above and then twice with 25 ml of fresh chloroform. The extracts were neutralised with $21.00 + 1.40 + 0.02 = 22.42$ ml of N-NaOH. As the sodium salt of the acid extracted was soluble in chloroform (see below), the layers were acidified together, shaken, separated, and the aqueous layer extracted a further twice with chloroform. Most of the chloroform was distilled off, and the remainder, 15 ml, treated with 50 ml of ether, which precipitated 7.60 g of crystals, m.p. 151—152° (efferv.), equiv. 431.6. Recrystallisation from 25 ml of ethanol yielded pure bisbenzenesulphonylamidine of cyclohexanesulphinic acid as small, hard, and glistening bipyramids of m.p. 154–155° (efferv.).

For $C_6H_{11} \cdot S(:NSO_2C_6H_5)NHSO_2C_6H_5 = C_{18}H_{22}O_4N_2S_3$ (426.56):

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| calc.: | C | 50.68 | H | 5.20 | N | 6.57 | S | 22.55 % | equiv. | 426.6 |
| found: | » | 50.87 | » | 5.15 | » | 6.61 | » | 22.66 » | » | 429.9 |

The amidine was soluble in ethyl acetate and acetone, less so in chloroform, still less in the other common solvents. The solubility in boiling ether was 0.4 g per 100 ml, in boiling water 0.4 g per 100 ml, and in water at room temperature 0.2 g per 100 ml. As a rather strong acid it could be titrated with alkali using phenolphthalein or phenol red. The sodium salt could be extracted from its aqueous solution with ethyl acetate or chloroform, but did not crystallise. Recrystallisation of the amidine from chloroform sometimes yielded the same product, that was obtained from ethanol, but sometimes instead brittle needles with one mole of chloroform, that was given off if the crystals were left in the air, so the m.p. was not affected. In a sealed capillary a specimen turned brown at 120° and at higher temperatures formed a black, opaque mass.

In accordance with previous observations (22) the amidine was rather stable. When it was heated in water or in neutral or alkaline aqueous solution for 4 hours in a water bath no decomposition occurred. When heated in the same way with 2 N-HCl it was slightly decomposed with a formation of benzenesulphonamide, sulphur dioxide, and unidentified sticky products, but most of the amidine was recovered unchanged.

When the amidine was heated above the melting point a small amount of cyclohexene distilled off, but no sulphur dioxide was formed. The residue was a black, partly crystalline mass, from which benzenesulphonamide could be extracted with warm water. From the black residue, insoluble in water, nothing of interest was isolated.

The ether-chloroform mother-liquor of the crude amidine obtained above was neutralised with 4.80 ml of N-NaOH. Acidification of the neutral extract solution precipitated an oil, which was taken up in ether, the ether neutralised with 3.4 ml of N-NaOH, and the oil reprecipitated as before. It did not crystallise when scratched or seeded with amidine. It was once again dissolved in ether, the solvent evaporated, and the residue dried in vacuo on a water bath to yield 0.9 g of a very viscous oil of the equiv. 358. Material from another experiment had the equiv. 370. The latter was analysed, the former used in some experiments described below.

For $C_{12}H_{17}O_4NS_2$ (303.39):

calc.: C 47.50 H 5.65 N 4.62 S 21.13 %
found: » 47.24 » 5.33 » 5.06 » 20.77 »

In order to characterise the compound (or mixture) it was subjected to treatment in alkaline and acid solution. A turbid, neutral solution was obtained by dissolving 0.19 g of the substance in 5.40 ml of 0.1 N-NaOH. The solution was cleared with ether and then made akaline with 2.12 ml of N-NaOH. The alkaline solution was heated for 4 hours on a water bath. It was then allowed to cool down and was neutralised with 1.10 ml of 2 N-HCl. Half of the solution was distilled off, the distillate made 2 N with respect to HCl, and was then treated with a few ml of 0.2 % solution of 2.4-dinitrophenylhydrazine in 2 N-HCl. Yellow crystal flakes of m.p. 158–160° resulted, after recrystallisation from methanol identified as the corresponding hydrazone of cyclohexanone of m.p. and mixed m.p. 158–160°. The residue from the distillation deposited some crude benzenesulphonamide of m.p. 150–152°, and a further portion was extracted from the filtrate with ethyl acetate. Acidification of the extracted solution made some sulphur dioxide free and precipitated a small amount of an oil, that by and by solidified.

The remainder of the material was dissolved in 6 ml of ethanol and treated with 3 ml of conc. hydrochloric acid. The clear solution was heated on a water bath for 1.5 hours. Nine ml were distilled off and treated with 2,4-dinitrophenylhydrazine, but failed to give a precipitate. The residue from the distillation was extracted with ether. The extract was neutralised with 1.25 ml of N-NaOH. Evaporation of the ether yielded 0.38 g of crude benzenesulphonamide, recrystallised from water, of m.p. 150–152°. The neutral, aqueous solution was acidified to precipitate an oil which, on being scratched, crystallised and had m.p. 145–165°. Recrystallisation from benzene-light petroleum yielded a small amount of glistening, soft needles, m.p. 166–171°, mixed m.p. with cyclohexanesulphonylbenzenesulphonamide 168–172° (see p. 211). The equiv. was 300, calc. 303.4. The mother-liquor of the crude product smelt of cyclohexanone and gave with 2,4-dinitrophenylhydrazine a small amount of the expected hydrazone of m.p. 154–156°. As cyclohexanesulphonylbenzenesulphonamide does not yield cyclohexanone under these conditions the material must have contained some unidentified compound.

The aqueous solution, that resulted from the extraction of the bisbenzenesulphonylamidine of cyclohexanesulphinic acid and the foregoing material with chloroform, was made up to 200.0 ml. With aqueous barium chloride 25 ml of it yielded 0.0906 g of barium sulphate. The main portion of the solution was neutralised with 23.96 ml of N-NaOH and evaporated to dryness at the pump on a water bath. The remaining crystalline cake was extracted twice with 25 ml of ethyl acetate, which dissolved an infinitesimal amount, and then with 50 ml of absolute ethanol. Free evaporation of the solvent left 0.08 g of crystalline material, that was recrystallised from 0.2 ml of water, but no crystal flakes of sodium cyclohexanesulphonate were obtained.

The results of the experiment may be summarised in the following way. From 1 mole of dicyclohexyl disulphide and 4 moles of chloramine-B were obtained 1.05 mole (this figure is somewhat too low on account of an accident) of benzenesulphonamide, 3.90 moles of chloride ion, 1.17 mole of bisbenzenesulphonylamidine of cyclohexanesulphinic acid, 0.32 mole of a mixture containing the foregoing compound, cyclohexanesulphonylbenzenesulphonamide, and an unknown compound, which in alkaline solution yielded cyclohexanone, benzenesulphonamide, and sulphur dioxide, 0.214 mole of sulphuric acid, an unknown amount of sulphur, and probably cyclohexene. The production of acid compounds was directly determined to 1.92 equivalents and calculated from the products isolated to 1.92 equiv. These results account for 85 % of the sulphur in the disulphide, 75 % of the cyclohexane-nucleus, and 93 % of the benzenesulphonamide-radical.

### b. Dicyclohexyl disulphide and chloramine-B in pyridine-water

Preliminary experiments showed that also in this case 4 moles of chloramine were consumed per mole of disulphide.

Eighty ml of M aqueous chloramine-B were added in portions to a solution of 4.61 g of dicyclohexyl disulphide in 200 ml of pyridine. A clear, yellow solution of pH 6.5 resulted. After some minutes a drop of it did not oxidise aqueous potassium iodide. It was neutralised (phenol red) with 37.40 ml of N-NaOH. The end point was not very sharp. The solution was then evaporated at the water pump on a water bath nearly to dryness. In another experiment the vapours were condensed and after acidification tested for cyclohexanone with 2,4-dinitrophenylhydrazine. No precipitate formed. The oily residue was treated with 70 ml of ether and 100 ml of water. The aqueous layer was then extracted 6 times with 70 ml of ether. The combined ethereal solutions were then evaporated to yield 3.38 g of crude benzenesulphonamide of m.p. 142–149°, after recrystallisation from water, of m.p. 151–153°. A small amount of brownish oil remained undissolved in the water. It had $n_D^{20}$ 1.5553 and may have contained unreacted disulphide.

The aqueous layer was contaminated with a white crystal powder, which was sucked off and consisted of 1.05 g of microscopic rods of m.p. 208° (efferv.).

For $C_{17}H_{20}O_3N_2S_2$ (364.47):

calc.: C 56.02 H 5.53 N 7.69 S 17.59 %
found: » 55.79 » 5.46 » 7.67 » 17.74 »

The material was soluble in warm glacial acetic acid, warm ethanol, and warm methanol, but insoluble in other organic solvents. It was insoluble in water, N-NaOH, and dilute mineral acids, but very soluble in conc. HCl and conc. $H_2SO_4$. A solution of 0.4 g in 4 ml of conc. HCl was diluted with 25 ml of water and then yielded 0.35 g of crystal needles of recovered material, m.p. 211° (efferv.), which in the air became somewhat yellowish. To get some insight into the constitution of the compound, 0.40 g was dissolved in 2 ml of conc. HCl, and the solution refluxed for half an hour. The solution was cooled and it then deposited a thick crystal porridge, which consisted of 0.10 g of glistening flakes of benzenesulphonamide, m.p. 151–153°. The mother-liquor (and washings) were extracted twice with ether, and the ether neutralised with 3 drops of N-NaOH. Half the extracted solution was distilled off and the distillate, which smelt of sulphur dioxide, extracted twice with ether. After neutralisation it was combined with the neutral, ethereal extract obtained above, and evaporated. The oil left, smelt of cyclohexanone and with 2,4-dinitrophenylhydrazine in 2 N-HCl yielded the expected hydrazone, m.p. 152–156°, after recrystallisation from methanol, m.p. 157–159°, undepressed on admixture with authentic cyclohexanone 2,4-dinitrophenylhydrazone. The residue from the distillation, which was brownish, was neutralised (phenolphthalein), and it then smelt of pyridine. It was taken up in ether and the solution dried with sodium hydroxide. Evaporation of the solvent yielded a very small residue, which was dissolved in 3 ml of a warm, saturated solution of picric acid in methanol. Cooling afforded needles of pyridine picrate of m.p. 167–168°. The m.p. found in the literature is e. g. 165–166° (58).

The filtrate from the crude product of this compound was made up to 150.0 ml. It was examined as described in the foregoing section. Five ml contained chloride ions corresponding to 25.53 ml of 0.1 N-$AgNO_3$. The chloroform extract was neutralised with 27.90 ml of N-NaOH, the whole mixture reacidified and shaken, the chloroform layer evaporated and precipitated with ether to yield 11.40 g of bisbenzenesulphonylamidine of cyclohexanesulphinic acid, m.p. 154–156°, equiv. 428.5. The chloroform-ether mother-liquor was neutralised with 1.20 ml of N-NaOH, and acidification of the neutral extract afforded a small amount of material that partly crystallised. The solution, extracted with chloroform, was made up to 200.0 ml, and 25 ml thereof treated with aqueous barium chloride to give 0.1366 g of barium sulphate. The main portion was evaporated to dryness as described and the residue neutralised with some N-NaOH and evaporated again. The crystalline residue was extracted twice with ether, which practically did not dissolve anything, and then twice with 50 ml of boiling, absolute ethanol. Evaporation of the solvent left 0.38 g of crystal flakes of somewhat impure sodium cyclohexanesulphonate.

For $C_6H_{11} \cdot SO_3Na$, $H_2O$ = $C_6H_{13}O_4SNa$ (204.22):

|  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| calc.: | C | 35.29 | H | 6.41 | S | 15.70 | Na | 11.26 | $H_2O$ | 8.82 | % |
| found: | » | 33.19 | » | 5.73 | » | 14.54 | » | 12.75 | » | 8.12 | » |

In this case 1 mole of dicyclohexyl disulphide and 4 moles of chloramine-B yielded 1.08 mole of benzenesulphonamide, 3.83 moles of chloride ion, 1.40 mole of bisbenzenesulphonylamidine of cyclohexanesulphinic acid, 0.14 mole of a compound (probable formula, see p. 177) which on acid hydrolysis yielded cyclohexanone, pyridine, benzenesulphonamide, and sulphur dioxide, 0.11 mole of cyclohexanesulphonic acid, and 0.24 mole of sulphuric acid. The production of acid compounds was directly determined to 1.87 equivalents and calculated from the amounts isolated to 1.99 equiv. These results account for 95 % of the disulphide sulphur, 82 % of the cyclohexane-nucleus, and 100 % of the benzenesulphonamide-radical. Cyclohexene was probably also formed. The deficiency of recovered chlorine and the acids directly titrated might depend on chlorination of pyridine with chloramine. This, and the formation of cyclohexene, was confirmed in the experiment now to be described.

### 3. Cyclohexyl thiolacetate and chloramine-B

This reaction proceeded more slowly than the foregoing ones. The competing reaction between chloramine and the solvent, noticeable in the reactions with dicyclohexyl disulphide, became more prominent, so the end point of the reaction was not so sharp. It was not found practicable to carry out the reaction in acetone-water as it was difficult to observe the end point in the neutralisation of the reaction mixture and the reaction velocity seemed to be highly dependent on pH. The reaction was therefore studied in pyridine-water.

A solution of 25.27 g of chloramine-B (oxidation equiv. 121.5) in 90 ml of water was added in 3 portions to a solution of 6.33 g of cyclohexyl thiolacetate in 200 ml of pyridine. The reaction mixture was cooled with water. The somewhat turbid mixture deposited a small, aqueous bottom layer. After 15 minutes a drop of it did not oxidise aqueous potassium iodide. The solution was neutralised with 73.30 ml of N-NaOH (phenol red, end point unsharp). The following day 27 ml were distilled off, neutralised with a few drops of N-NaOH, and then treated with 5.00 ml of N-NaOH. After one hour it was neutralised with 2.40 ml of 2 N-HCl and then extracted with 50 ml of ether. The ethereal layer was washed twice with water and then with dilute hydrochloric acid. A small amount of cyclohexanethiol was removed with mercuric chloride in ether. The mother-liquor from the mercaptide precipitate was distilled off on a water bath and the distillate treated with 4 × 5 ml of 0.1 N bromine in glacial acetic acid. After the last addition the solution remained yellow. It was decolourised with aqueous sulphur dioxide, washed with water and finally neutralised with alkali. The neutral, ethereal solution was dried with calcium chloride and evaporated to yield 0.2 g of an oily residue of the typical odour of 1,2-dibromocyclohexane. It contained 60.7 % Br and had $n_D^{20}$ 1.5420. For the pure compound the Br-content is 66.06 % and $n_D^{20}$ is given as 1.5530 (59).

The remainder of the reaction mixture was distilled off nearly to dryness on a water bath at the pump. The distillate, 328 ml, was analysed for chlorine and unreacted thiolacetate. A test for cyclohexanone was negative. The chlorine content corresponded to 8.7 ml of N-$AgNO_3$. The determination was rather uncertain, as it was made on a sample of 2 ml. For the determination of the thiolacetate a sample of the distillate was hydrolysed with excess of N-NaOH, then acidified with acetic acid, and titrated with iodine. The content was estimated to 4.13 millimoles of cyclohexyl thiolacetate, which was also noticed by its odour. Some unsuccessful attempts were made to isolate a chlorine-containing compound.

The brownish residue from the distillation was treated with 200 ml of water and 150 ml of ether. The clear, aqueous layer was then extracted with the ether in an efficient, continuous extractor. A brownish, crystalline extract was obtained. It smelt of pyridine, and was extracted twice with 30 ml of light petroleum, which dissolved 0.2 g of an oil that contained 10.24 % of chlorine. The undissolved material, 5.83 g, melted at 135–143°. Recrystallisation from water yielded benzenesulphonamide of m.p. 151–153° but also some acid material corresponding to 3.9 ml of N-NaOH, part (0.63 g) of which was identified as bisbenzenesulphonylamidine of cyclohexanesulphinic acid, m.p. 146–152° (efferv.), equiv. 433. It probably also contained some acetylbenzenesulphonamide (see below). These acid compounds might have been extracted in the form of pyridinium salts.

The extracted solution was made up to 250 ml. The content of chloride ions was determined as before and corresponded to 39.14 ml of 0.1 N-$AgNO_3$ in 10 ml of the solution.

The main portion was acidified with 15.00 ml of 5 N-HCl. A sticky mass separated. It soon crystallised and weighed 10.05 g. Extraction 4 times with 25 ml of ether left 9.00 g undissolved. This material melted at 146–151° (efferv.) and had the equiv. 433.7. Recrystallisation from ethanol yielded pure bisbenzenesulphonylamidine of cyclohexanesulphinic acid, m.p. 152–154° (efferv.), equiv. 428.3.

The ethereal extracts were neutralised with $1.30 + 1.00 + 0.90 + 0.50 = 3.70$ ml of N-NaOH. Acidification, extraction with chloroform, and evaporation afforded 0.79 g of crystalline material, m.p. 90–108°, equiv. 245.9.

The mother-liquor from the crude amidine (10.05 g) was extracted 4 times with 30 ml of chloroform and the extracts neutralised with $8.60 + 2.40 + 1.30 + 0.70 = 13.00$ ml of N-NaOH. An infinitesimal amount of undissolved crystal powder was filtered off. It melted at 206° (efferv.), was soluble in conc. HCl, and thus consisted of the compound described on p. 217. The combined chloroform and water layers were acidified with 3 ml of 5 N-HCl, shaken, and the chloroform layer freely evaporated to yield 2.82 g of crystalline residue of m.p. 100–115° and equiv. 222.0. Together with the above-mentioned material, equiv. 245.9, it was purified by recrystallisation from benzene, ethanol-water, and finally benzene to yield pure acetylbenzenesulphonamide, m.p. 123–125°. **Böhme** and **Wagner** (60) report the m.p. 126°.

For $C_6H_5SO_2NHCOCH_3 = C_8H_9O_3NS$ (199.22):

| | | | | | | |
|---|---|---|---|---|---|---|
| calc.: | C 48.23 | H 4.55 | N 7.03 | S 16.09 % | equiv. 199.2 | |
| found: | » 48.67 | » 4.55 | » 7.22 | » 16.06 » | » 201.3 | (phenolphthalein) |

The solution that had been extracted with chloroform was then extracted with ether in the above-mentioned extractor, which extracted acid material corresponding to 17.25 ml of N-NaOH, identified as acetic acid by means of its silver salt, which was precipitated from the neutral solution with silver nitrate. Recrystallisation from water yielded the almost pure salt, containing 63.51 % Ag, calc. 64.63 %.

The extracted solution was made up to 300 ml and 25 ml precipitated with aqueous barium chloride to yield 0.1636 g of barium sulphate. The main portion was neutralised with 16.30 ml of N-NaOH and evaporated to dryness as before. No sodium cyclohexanesulphonate was found in the residue.

The results might be summarised thus. One mole of cyclohexyl thiolacetate and 2.92 moles of chloramine-B yielded (in substance or by calculation) 0.84 mole of benzenesulphonamide, 2.74 moles of chloride ion, 0.26 mole of organic chlorine compounds (this figure was rather uncertain and seems too high), 0.71 mole of bisbenzenesulphonylamidine of cyclohexanesulphinic acid, 0.49 mole of acetylbenzenesulphonamide, 0.51 mole of acetic acid, 0.25 mole of sulphuric acid, traces of the compound discussed on p. 177, and an unknown amount of cyclohexene. The production of acid compounds was directly determined to 2.06 equivalents and calculated from the amounts isolated to 2.20 equiv. These results account for 96 % of the thiolacetate sulphur, 71 % of the cyclohexane-nucleus, 100 % of the acetyl-radical, and 94 % of the benzenesulphonamide-radical.

### 4. Cyclohexyl thiolbenzoate and chloramine-B

This reaction proceeded much more slowly than the foregoing ones, so it could not be investigated so closely. In a small scale experiment the following products were identified: benzenesulphonamide (m.p. 149–152°), bisbenzenesulphonylamidine of cyclohexanesulphinic acid (a very small amount of m.p. 156–157° (efferv.)), benzoylbenzenesulphonamide (m.p. 147–148°, equiv. 258, calc. 261.3; Wallach (61) reports the m.p. 147°), benzoic acid (m.p. 118–120°), and sulphuric acid.

### 5. Cyclohexyl cyclohexanethiolsulphonate and chloramine-B

A solution of 0.26 g of the ester in 10 ml of acetone was mixed with a solution of 0.49 g of chloramine-B in 5 ml of water. A clear solution formed and no heat of reaction was observed. The pH remained unchanged at 7 and a drop of the mixture oxidised aqueous potassium iodide. After some minutes the solution was diluted with water and the initial material recovered in quantity by extraction with ether.

The reaction, if any, thus must be slow. Under these circumstances the reaction between acetone and chloramine should not be neglected, so another experiment was made with pyridine as a solvent. A solution of 0.26 g of cyclohexyl cyclohexanethiolsulphonate in 10 ml of pyridine was mixed with a solution of 1.00 g of chloramine-B in 5 ml of water. The next day a drop of the solution still oxidised aqueous potassium iodide. It was diluted with 25 ml of water, neutralised (phenol red, unsharp end point) with 1.30 ml of N-NaOH, and extracted with 50 ml of light petroleum. The extract was freely evaporated to yield 0.06 g of an oily residue which, when seeded with the thiolsulphonate, partly crystallised, and after draining on porous tile had m.p. 34–36°.

The extracted pyridine-water solution was extracted 6 times with 50 ml of ether, this evaporated, and the pyridine residue freely evaporated to yield 0.4 g of crude benzenesulphonamide, after recrystallisation of m.p. 151–153°.

Acidification of the extracted solution gave a turbid solution that was extracted twice with ether and once with chloroform. Clear layers were formed in each case and on free evaporation the ethereal solutions yielded 0.22 + 0.05 g of crystalline residue of m.p. 90–115°, whereas the chloroform extracted practically nothing. The final solution was treated with aqueous barium chloride to yield 0.1090 g of barium sulphate.

The ethereal extract was recrystallised from 6 ml of benzene. Some benzenesulphonamide of m.p. 147–152° separated and the mother-liquor was treated with an equal volume of light petroleum, which precipitated an unhomogeneous crystalline product. The supernatant liquid was decanted and the crystals extracted with 6 + 2 + 2 ml of ether, which left some small, hard, glistening crystals of bisbenzenesulphonylamidine of cyclohexanesulphinic acid undissolved, m.p. and mixed m.p. 150–152° (efferv.).

The ethereal extract was precipitated with light petroleum from its solution in a few ml of benzene to yield cyclohexanesulphonylbenzenesulphonamide, m.p. 164–168°, on admixture with authentic material (see p. 211), m.p. 166–170°.

REFERENCES. 1. **B. Weibull:** Ark. Kemi Min. Geol. *23 A*, no. 18 (1946). — 2. **J. L. Gleave, E. D. Hughes,** and **C. K. Ingold:** J. Chem. Soc. *1935*, 236. — 3. **B. Holmberg:** Ark. Kemi Min. Geol. *12 A*, no. 11 (1936). — 4. ——: Ark. Kemi Min. Geol. *12 A*, no. 28 (1938). — 5. **E. A. Letts:** Trans. Roy. Soc. Edinburgh *28*, 591 (1878). — 6. **A. Delisle:** Ber. *25*, 2450 (1892). — 7. **G. Carrara:** Gazz. chim. ital. *23*, I, 506 (1893). — 8. **B. Holmberg:** Ark. Kemi Min. Geol. *21 B*, no. 7 (1945). — 9. **B. Holmberg** and **E. Schjånberg:** Ark. Kemi Min. Geol. *15 A*, no. 20 (1942). — 10. **K. Jönsson:** Diss., Lund 1929. — 11. **B. Holmberg:** Ark. Kemi Min. Geol. *14 A*, no. 8 (1940). — 12. **N. Kharasch, S. J. Potempa,** and **H. L. Wehrmeister:** Chem. Reviews *39*, 269 (1946). — 13. **B. Weibull:** Ark. Kemi *3*, no. 25 (1951). — 14. **L.-J. Simon:** C. R. Acad. Sci., Paris *137*, 855 (1903). — 15. **S. Smiles** and **D. T. Gibson:** J. Chem. Soc. *125*, 176 (1924) and later papers in the same series. — 16. **P. Fitger:** Ber. *54*, 2952 (1921). — 17. **W. Autenrieth:** Ann. *254*, 222 (1889). — 18. **F. G. Mann** and **W. J. Pope:** J. Chem. Soc., *121*, 1052 (1922). — 19. **A. Tananger:** Ark. Kemi Min. Geol. *24 A*, no. 10 (1947). — 20. **E. Baumann** and **G. Walter:** Ber. *26*, 1125 (1893). — 21. **C. M. Suter:** The Organic Chemistry of Sulfur. New York 1944. — 22. **S. G. Clarke, J. Kenyon,** and **H. Phillips:** J. Chem. Soc. *1930*, 1225. — 23. **J. R. Alexander** and **H. McCombie:** J. Chem. Soc. *1932*, 2087. — 24. **G. Bulmer** and **F. G. Mann:** J. Chem. Soc. *1945*, 666. — 25. **O. Foss:** Acta Chem. Scand. *1*, 307 (1947). — 26. **A. D. Mitchell:** British Chemical Nomenclature. London 1948. — 27. **W. Borsche** and **W. Lange:** Ber. *39*, 397 (1906). — 28. **K. H. Meyer** and **W. Hohenemser:** Helv. Chim. Acta *18*, 1061 (1935). — 29. **D. Strömholm:** Diss., Uppsala 1899. — 30. **N. Hellström** and **B. Holmberg:** Ark. Kemi Min. Geol: *12 A*, no. 2 (1935). — 31. **N. Hellström:** Z. physik. Chem. *A 177*, 337 (1936). — 32. **B. Holmberg** and **E .Schjånberg:** Ark. Kemi Min. Geol. *15 A*, no. 23 (1942). — 33. **G. Ciamician** and **P. Silber:** Ber. *29*, 485 (1896). — 34. **E. Schulze:** Z. für Chemie *8*, 77 (1865). — 35. **S. Blackburn** and **F. Challenger:** J. Chem. Soc. *1938*, 1878. — 36. **W. F. Faragher, J. C. Morrell,** and **S. Comay:** J. Amer. Chem. Soc. *51*, 2774 (1929). — 37. **C. F. H. Allen:** J. Amer. Chem. Soc. *52*, 2957 (1930). — 38. **J. M. Lovén:** Ber. *29*, 1140 (1896). — 39. **D. Barnard, J. M. Fabian,** and **H. P. Koch:** J. Chem. Soc. *1949*, 2442. — 40. **F. Eisenlohr:** Z. physik. Chem. *75*, 585 (1911). — 41. **W. Strecker** and **R. Spitaler:** Ber. *59*, 1754 (1926). — 42. **J. von Braun** and **K. Weissbach:** Ber. *63*, 2842 (1930). — 43. **D. W. Cowie** and **D. T. Gibson,** J. Chem. Soc. *1934*, 48. — 44. **A. Elbers:** Ann. *227*, 353 (1885). — 45. **E. Simon** and **C. Neuberg:** Biochem. Z. *232*, 479 (1931). — 46. **F. D. Chattaway:** J. Chem. Soc. *87*, 145 (1905). — 47. **J. Stenhouse:** Ann. *140*, 294 (1866). — 48. **W. Borsche** and **W. Lange:** Ber. *38*, 2766 (1905). — 49. **H. Gilman** and **L. L. Heck:** J. Amer. Chem. Soc. *50*, 2229 (1928). — 50. **J. I. Cunneen:** J. Chem. Soc. *1947*, 36. — 51. **J. M. Sprague** and **T. B. Johnson:** J. Amer. Chem. Soc. *59*, 1837 (1937). — 52. **I. Kolker** and **A. Lapworth:** J. Chem. Soc. *127*, 312 (1925). — 53. **E. F. Landau:** J. Amer. Chem. Soc. *69*, 1219 (1947). — 54. **I. Hedlund:** Diss., Uppsala 1942. — 55. **B. Helferich** and **A. Flechsig:** Ber. *75*, 532 (1942). —

56. **M. L. Crossley, E. H. Northey,** and **M. E. Hultquist**: J. Amer. Chem. Soc. 62, 1415 (1940). — 57. **A. Wolkow**: Z. für Chemie *13,* 323 (1870). — 58. **P. Brandes** and **C. Stoehr**: J. prakt. Chem. [2] *54,* 488 (1896). — 59. **F. Swarts**: Bull. Acad. Belg., Cl. Sci. [5] *22,* 924 (1936). — 60. **H. Böhme** and **J. Wagner**: Ber. *75,* 614 (1942). — 61. **O. Wallach**: Ann. *214,* 211 (1882).

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# On cyclohexylthio-derivatives of acrylic, fumaric, and maleic acid

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With 2 figures in the text

## I. Introduction and survey

In another paper (1) the author described the formation of a cyclohexylthioacrylic acid by degradation of cyclohexylsulphinylsuccinic acid. In order to prove its structure and as the properties of compounds with sulphur linked to an unsaturated carbon atom are little known, it was considered worth while to prepare some unsaturated cyclohexylthio-carboxylic acids. In this paper the properties of the above-mentioned cyclohexylthioacrylic acid is described together with similar compounds prepared by the addition of cyclohexanethiol to propiolic and acetylenedicarboxylic acid.

The first representative of a compound of this type was obtained by ESCALES and BAUMANN (2), who prepared a 2-phenylthiocrotonic acid by decomposition of 2,2-bisphenylthiobutyric acid. Their work was continued by AUTENRIETH (3), who prepared all the possible isomers of 1- and 2-phenylthiocrotonic acid and 2-ethylthiocrotonic acid by means of the sodium salts of the corresponding chlorocrotonic acids and thiols. His allocation of stereoisomers now seems obsolete. He found that the 2-isomers were decomposed by heating with acid or alkali with thiol formation, but the 1-isomers were more stable.

Reactions between thiols and acetylene-carboxylic acids were reviewed by JOHNSON (4). Those of RUHEMANN and STAPLETON (5) are of interest in this connection. They added sodium phenyl mercaptide to the ethyl esters of phenylpropiolic and acetylenedicarboxylic acid. In the former case only one product was obtained, in the latter one mono-adduct, assumed to be the fumaric acid derivative, and a di-adduct.

Recently BAKER and QUERRY (6) added 4-acetaminobenzenethiol to propiolic and acetylenedicarboxylic acid in the presence of ammonia and obtained only one mono-adduct, in the latter case considered to be the fumaric acid derivative. The product formed by hydrolysing the adduct prepared from the diethyl ester of acetylenedicarboxylic acid was isomeric with the one obtained directly from the acid, and thus was considered to be the maleic acid derivative.

The only previous investigation strictly comparable with the present one is that of OWEN and SULTANBAWA (7). Using toluene-ω-thiol they obtained with propiolic acid the stereoisomeric 2-benzylthioacrylic acids. With thiolacetic acid the corresponding compounds were formed. The reaction between methyl propiolate and thiolacetic acid in addition afforded the 1,2-di-adduct. Thiolacetic acid and acetylenedicarboxylic acid (in ethyl acetate) or its ester, yielded the stereoisomeric symmetric di-adducts.

**1-Cyclohexylthioacrylic acid.** As previously described this compound was formed when heating a neutral or acid solution of cyclohexylsulphinylsuccinic acid. As will be described later on, it was also formed, in a small yield, by heating an acid, aqueous glycolic solution of cyclohexylthiofumaric acid.

The acid was rather unstable. When kept for some weeks in the air it yielded an oil and smelt of cyclohexanethiol. The S-benzyl-*iso*-thiuronium-salt was stable and well defined. When 1-cyclohexylthioacrylic acid was heated in aqueous ethanolic solution it was decomposed with a formation of cyclohexanethiol and pyruvic acid:

$$1.\quad CH_2 = C(SC_6H_{11})COOH + H_2O \rightarrow C_6H_{11}SH + CH_3COCOOH.$$

The acid hydrolysis of an unsaturated sulphide to thiol and ketone was observed by RUHEMANN (5). On the other hand the compound was stable in neutral or alkaline solution. In warm aqueous ethanolic solution it rapidly added a molecule of cyclohexanesulphinic acid:

$$2.\quad CH_2 = C(SC_6H_{11})COOH + C_6H_{11} \cdot SO_2H \rightarrow$$
$$\rightarrow C_6H_{11} \cdot SO_2 \cdot CH_2CH(SC_6H_{11})COOH.$$

In alkaline solution the last-formulated acid, 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid was decomposed with formation of salts of the original acids. With potassium permanganate in neutral solution it was oxidised to 1,2-biscyclohexylsulphonylpropionic acid, which on heating above the melting point (133°) was decarboxylated to 1,2-biscyclohexylsulphonylethane (m.p. 156.5–159°). The same compound was obtained by oxidation of 1,2-biscyclohexylthioethane, prepared from 1,2-dibromoethane and sodium cyclohexyl mercaptide.

These reactions unequivocally determine the structure of 1-cyclohexylthioacrylic acid and the compounds prepared from it.

**2-Cyclohexylthioacrylic acids.** In a mixture of equimolar amounts of cyclohexanethiol and propiolic acid there occurred, at room temperature, no noticeable reaction. A rapid reaction was started either by heating or by the addition of a catalytical amount of conc. sulphuric acid. In the former case the main product was a 2-cyclohexylthioacrylic acid (m.p. 151–154°), provisionally named A-acid, which was easily purified by recrystallisation from ethanol. As by-products there were formed small amounts of the stereoisomer, named B-acid, 2,2-biscyclohexylthiopropionic acid, and 1,1-cyclohexylthioethane. The two last-mentioned compounds did not crystallise, but by oxidation the corresponding, well crystallising sulphones were prepared. The biscyclohexylsulphonylpropionic acid obtained (m.p. 171–172°) was not identical with the one previously described. The third possible isomer, 1,1-biscyclohexylsulphonylpropionic acid, is not stable (compare ESCALES and BAUMANN (8)). Pyruvic acid and cyclohexanethiol on heating with mineral acid yielded the non-crystallising 1,1-biscyclohexylthiopropionic acid, which on oxidation immediately lost carbon dioxide and afforded 1,1-biscyclohexylsulphonylethane (m.p. 187–188°), identical with the above-mentioned but not with the one described in the foregoing paragraph. Thus the structure of these compounds is proved and likewise the position of the cyclohexylthio-group in the unsaturated acid.

In the latter case, when the addition-reaction was started with catalytical amounts of conc. sulphuric acid, the reaction products formed a mixture of the stereoisomeric 2-cyclohexylthioacrylic acids, with excess of the B-acid, and considerable amounts of 2,2-biscyclohexylthiopropionic acid, characterised as the one obtained in the thermal reaction and identical with it. The mixture of isomers was separated by combining recrystallisation with a procedure of extraction. According to the latter, a solution of the mixture in ether was half-neutralised with aqueous alkali. The B-acid was enriched in the ether, whereas the A-acid preferably remained in the aqueous phase. This procedure was used in systematic fractionation. The enrichment was rather rapid, and the pure isomers were finally obtained by recrystallisation. The m.p. of the B-acid was 112–114°. The same procedure was applied to separate the B-acid formed in the thermal reaction.

The chemical properties of the isomers were almost identical. In neutral and alkaline solution no decomposition, but possibly slight isomerisation, was observed. In warm aqueous propanolic solution, the compounds were decomposed, more rapidly when made acid, with formation of cyclohexanethiol, acetaldehyde and carbon dioxide, the latter compounds being formed by decomposition of formylacetic acid, postulated as an intermediate, (compare 9):

$$3.\quad C_6H_{11}S \cdot CH = CH \cdot COOH + H_2O \rightarrow C_6H_{11}SH + OHC \cdot CH_2 \cdot COOH \rightarrow$$
$$\rightarrow C_6H_{11}SH + CH_3CHO + CO_2\,.$$

In warm aqueous ethanolic solution the A-acid did not add cyclohexanesulphinic acid. On account of scarcity of material no experiment was made with the B-acid. When the isomers were melted and kept at 155° for half an hour their melting points were depressed, that of the A-acid appreciably, that of the B-acid only slightly. This may indicate isomerisation of the A-acid at least.

**Cyclohexylthiofumaric acid.** In the course of a few days, at room temperature, cyclohexanethiol reacted almost quantitatively with acetylenedicarboxylic acid in glacial acetic acid. The main product was cyclohexylthiofumaric acid. Besides, there was formed a small amount of cyclohexylthiomaleic anhydride (see below), which settles the structure of the products.

On heating cyclohexylthiofumaric acid in aqueous glycol it was decomposed. Final products were cyclohexanethiol, pyruvic acid, and carbon dioxide, the latter compounds being formed by decomposition of oxaloacetic acid, postulated as an intermediate (compare 10). Small amounts of 1-cyclohexylthioacrylic acid were formed in a side reaction or as an intermediate product, besides which isomerisation to cyclohexylthiomaleic acid, isolated as the anhydride, occurred:

$$\begin{tikzcd}
\text{4 a.}\ C_6H_{11}\cdot S\cdot C(COOH)=CHCOOH+H_2O \arrow[d] \\
C_6H_{11}\cdot SH+HOCO\cdot CO\cdot CH_2\cdot COOH \arrow[d] \\
C_6H_{11}\cdot SH+HOCO\cdot CO\cdot CH_3+CO_2 \\
\text{4 b.}\ C_6H_{11}\cdot S\cdot C(COOH)=CHCOOH \arrow[d] \\
C_6H_{11}\cdot S\cdot C(COOH)=CH_2+CO_2 \arrow[uu, bend right, "H_2O"']
\end{tikzcd}$$

In neutral and alkaline solution the compound was stable.

In warm aqueous ethanol, cyclohexylthiofumaric acid slowly added cyclohexanesulphinic acid, whereby the former was partly decomposed as described above. Reaction products were the above-mentioned 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid and (possibly) minute amounts of a cyclohexylthio-cyclohexylsulphonylsuccinic acid.

Heated above the melting point cyclohexylthiofumaric acid rapidly lost one mole of carbon dioxide. In the oily reaction mixture traces of cyclohexanethiol and pyruvic acid were identified. The main product consisted of a mixture of acids, that partly (about 30 %) crystallised. The residue solidified to some extent, but it was not possible to separate and purify it. The crystalline product, m.p. 133–135°, had the empirical composition of cyclohexylthioacrylic acid, but twice its molecular weight. Thus it must be either an unsaturated dimer of the same or a cyclobutane-derivative. In both cases many isomers are possible, dependent on which carboxyl group is split off and how the remaining radicals are combined. In warm, acid, aqueous propanolic solution the compound yielded a sticky product, from which nothing could be isolated. Cyclohexanethiol was not formed, not even when the heating was performed in the presence of mercuric chloride. In alkaline solution, on the other hand, cyclohexanethiol was formed together with a lemon-yellow, crystalline acid (m.p. 218–222°). The composition of this acid corresponded to a cyclohexylthio-cyclobutenedicarboxylic acid or a corresponding doubly unsaturated acyclic compound.

**Cyclohexylthiomaleic anhydride.** As mentioned above this compound was formed in a small yield together with cyclohexylthiofumaric acid by the addition of cyclohexanethiol to acetylenedicarboxylic acid. An aqueous solution of the sodium salt of cyclohexylthiomaleic acid was easily obtained by dissolving the anhydride in sodium hydroxide, or by neutralising its solution in dioxane or ether with alkali. The hydrolysis occurred almost momentarily, the end point was sharp. Acidification of the solution yielded an oil, which in the course of some days slowly gave crystals of the anhydride. The acid itself could not be obtained as crystals, and seemed to be unstable. When its ethereal solution was evaporated, the sticky residue gave crystals of the anhydride overnight.

In solutions cyclohexylthiomaleic acid reacted as the isomer. The anhydride was stable towards heating. With cyclohexanesulphinic acid there was obtained, besides 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid, traces of a cyclohexylthio-cyclohexylsulphonylsuccinic acid, isomeric with the one obtained from cyclohexylthiofumaric acid.

**The ionisation constants of the unsaturated acids.** As the chemical properties of the 2-cyclohexylthioacrylic acids did not differentiate the two isomers, and as the m.p. is not a reliable criterion for the allocation of *cis-trans*-isomers (by the benzyl-analogues, prepared according to similar methods by Owen and Sultanbawa (7), the relation between the m.p.'s was reversed) it was considered worth while to examine the ionisation constants in this respect. These were determined by potentiometric titration of the sodium salts of the acids with standard hydrochloric acid, the hydrogen activity being measured with a glass electrode using a Radiometer "pH Meter 22". The thermodynamical constants and solubility products of the monobasic acids were calculated by the method of Back and Steenberg (11), and for the dibasic acids similar methods were worked out. The first ionisation constants of these are less reliable on account of inherent weakness of the method of determination. For comparison the values of the ionisation constants of the unsubstituted acids are also cited from the literature, that of acrylic acid according to Moureu and Boutaric (12), those of fumaric and maleic acid according to Ashton and Partington (13).

*Table 1*

| Acid | p$K$ or p$K_1$ | p$K_2$ |
|---|---|---|
| Acrylic acid | 4.25 | |
| 1-Cyclohexylthioacrylic acid | 3.40 | |
| 2-Cyclohexylthioacrylic acid, A-acid | 4.95 | |
| 2-Cyclohexylthioacrylic acid, B-acid | 4.70 | |
| Fumaric acid | 3.02 | 4.32 |
| Maleic acid | 1.85 | 6.07 |
| Cyclohexylthiofumaric acid | 1.89 | 5.00 |
| Cyclohexylthiomaleic acid | 1.90 | 5.93 |

As is well known, substitution with sulphur increases the acidity of saturated carboxylic acids (see for example Larsson (14) concerning 1-alkylthioacetic acids and Lovén (15) concerning 2-(2-carboxyethylthio)-propionic acid, the only example found of a purely 2-substituted carboxylic acid). As seen above, the same was true for 1-cyclohexylthioacrylic acid, but both the 2-cyclohexylthioacrylic acids were weaker than the unsubstituted acrylic acid. This interesting fact may be attributed to a mesomeric interaction of the lone electron pairs of the sulphur atom with the double bond and the carboxyl group.

The ionisation constants of the dibasic acids are not directly comparable, because the unsubstituted acids are symmetric, the substituted ones unsymmetric. In the latter case the ionisation is determined by four constants, two for the first ionisation, $K_1'$ and $K_1''$, and two for the second, $K_2'$ and $K_2''$, which are related to those experimentally determined by the relations (see for example Söderbäck (16)):

$$K_1 = K_1' + K_1''$$

$$\frac{1}{K_2} = \frac{1}{K_2'} + \frac{1}{K_2''}.$$

In the case of symmetric acids one gets:

$$K_1' = K_1'' = K_1/2$$
$$K_2' = K_2'' = 2K_2.$$

In the case of a very unsymmetric acid one of the constants in each relation may be neglected in comparison with the other, and the experimentally determined constants in that case almost equal those related to the most acidic carboxyl group for the first ionisation ($K_1'$) and the least acidic one for the second ionisation ($K_2'$). As 1-cyclohexylthioacrylic acid is more than ten times as strong as the 2-cyclohexylthioacrylic acids, it seems plausible to make this assumption, and the constants to be compared are the following ones.

*Table 2*

| Acid | $pK_1'$ | $pK_2'$ |
|---|---|---|
| Fumaric acid | 3.32 | 4.02 |
| Maleic acid | 2.15 | 5.77 |
| Cyclohexylthiofumaric acid | 1.89 | 5.00 |
| Cyclohexylthiomaleic acid | 1.90 | 5.93 |

It is seen, that in this case too, the acidity of the carboxyl group adjacent to the sulphur atom was increased, whereas that of the other decreased. The decrease was larger for the fumaric acid derivative, which in this respect resembled the A-acid. If this implies similarity in configuration, the A-acid should be *cis*-2-cyclohexylthioacrylic acid and the B-acid *trans*-2-cyclohexylthioacrylic acid. The corresponding allocation was made by OWEN and SULTANBAWA (7) for the 2-benzylthioacrylic acids, using other criteria. Naturally neither allocation can be considered quite certain.

The nomenclature used is in accordance with MITCHELL (17), with the main exception that the prefix cyclo- is not italicised.

## II. 1-Cyclohexylthioacrylic acid

### 1. Simple derivatives and properties

1-Cyclohexylthioacrylic acid, m.p. 82–84°, was best prepared by heating a neutral solution of sodium cyclohexylsulphinylsuccinate as previously described (1). The compound slowly deteriorated in the air. The S-benzyl-*iso*-thiuronium salt was stable. A neutral solution of the sodium salt of the acid corresponding to 4.7 ml of N-NaOH in 50 ml of water was treated with a solution of 1.0 g of S-benzyl-*iso*-thiuronium chloride in 15 ml of water, affording 1.5 g of material of m.p. 161–163°. Recrystallisation from 80 ml of ethanol (95 % if not otherwise stated), yielded 1.1 g of crystal rods of m.p. 164–165° (efferv.) of the expected composition.

For $CH_2 = C(SC_6H_{11})COOH$, $H_2NC(:NH)SCH_2C_6H_5 = C_{17}H_{24}O_2N_2S_2$ (352.50):

| | | | | |
|---|---|---|---|---|
| calc.: | C 57.92 | H 6.86 | N 7.95 | S 18.19 % |
| found: | » 57.73 | » 6.91 | » 7.99 | » 18.24 » |

1-Cyclohexylthioacrylic acid was rather stable in alkaline solution. A neutral solution of 0.14 g of the acid in 7.56 ml of 0.1 N-NaOH was treated with 1.00 ml of N-NaOH and then heated for 2 hours on a water bath. Acidification precipitated 0.10 g of an almost unchanged product of m.p. 79–81°, equiv. 188.9, calc. 186.3.

In acid solution a rapid decomposition occurred. A neutral solution of 0.11 g of the acid in 5.96 ml of 0.1000 N-NaOH was acidified with 1.00 ml of 2 N-HCl and treated with 0.2 g of mercuric chloride in order to catch the cyclohexanethiol formed and to suppress side-reactions. Addition of 7 ml of ethanol gave a clear solution, which was heated on a water bath for 4 hours. The glistening flakes formed, weighing 0.16 g, were identified as chloromercury cyclohexyl mercaptide of the equiv. 354, calc. 351.3 (18). The filtrate therefrom was added to a hot solution of 0.2 g of 2,4-dinitrophenylhydrazine in 30 ml of 4 N-HCl, to yield 0.12 g of the 2,4-dinitrophenylhydrazone of pyruvic acid, after recrystallisation from ethyl acetate, of m.p. 215–216° (efferv.), not depressed on admixture with authentic material (m.p. 216° (19)).

The partition coefficient between ether and water was roughly determined by shaking a solution of about 0.3 g of the acid in 10.0 ml of ether, saturated with water, with 100 ml of water, saturated with ether. The aqueous layer was neutralised with 0.75 ml of 0.1004 N-NaOH, the ethereal, with 15.50 ml. If correction is made for the dissociation in the water (see p. 229) and the substance is assumed to be undissociated and unassociated in ether, the partition coefficient is found to be 425.

### 2. 1-Cyclohexylthio-2-cyclohexylsulphonylpropionic acid and 1,2-biscyclohexylsulphonylpropionic acid

To a solution of sodium cyclohexanesulphinate, prepared as described (1), and corresponding to 6.0 ml of N-NaOH, there was added 1.1 g of 1-cyclohexylthioacrylic acid, 3.0 ml of 2 N-HCl, and 7 ml of ethanol. The clear solution was then heated for 2 hours on a water bath. After cooling down, two 20 ml portions of ether extracted acids neutralised with 6.22 + 0.20 = = 6.42 ml of N-NaOH. Acidification with 3.5 ml of 2 N-HCl afforded an oil, which solidified to 1.60 g of crystals of m.p. 111–114°. Recrystallisation from 7 ml of ethyl acetate + 150 ml of light petroleum (b.p. 80–100° unless otherwise stated) afforded needles of pure 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid, m.p. 116–118°.

For $C_6H_{11}SO_2 \cdot CH_2CH(SC_6H_{11})COOH = C_{15}H_{26}O_4S_2$ (334.48):

| | | | | |
|---|---|---|---|---|
| calc.: | C 53.86 | H 7.84 | S 19.17 % | equiv. 334.5 |
| found: | » 53.69 | » 7.78 | » 18.99 » | » 334.8 |

Heating with warm alkali gave salts of the initial compounds. A neutral solution of 0.35 g of 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid in

10.39 ml of 0.1003 N-NaOH was evaporated to a small volume, treated with 1.10 ml of N-NaOH, and heated for 2 hours in a water bath. After being cooled down, the solution got neutral with 0.10 ml of 2 N-HCl. Acidification precipitated 0.17 g of crude 1-cyclohexylthioacrylic acid of m.p. 79–81° and equiv. 190.5, giving a crude S-benzyl-*iso*-thiuronium salt of m.p. 161–162°. The mother-liquor was extracted with three 15 ml portions of benzene, which were neutralised with 4.90 + 1.80 + 1.10 = 7.80 ml of 0.1003 N-NaOH. The neutral solution with S-benzyl-*iso*-thiuronium chloride yielded 0.15 g of the corresponding salt of cyclohexanesulphinic acid, m. p. and mixed m.p. 154–156°.

A neutral solution prepared from 1.11 g of 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid, 0.48 g of cryst. sodium carbonate, and 5 ml of water was oxidised with 14 ml of 0.95 N-$KMnO_4$ (calculated for reduction to $MnO_2$), carbon dioxide being bubbled through the cooled solution. Acidification of the filtrate from the manganese dioxide slime gave an oil, which solidified to a hard cake weighing 1.1 g, m.p. 127–130°. The pure 1,2-biscyclohexylsulphonylpropionic acid was obtained by recrystallisation from 15 ml of benzene + 15 ml of light petroleum and had m.p. 131–133° (efferv.).

For $C_6H_{11}SO_2 \cdot CH_2CH(SO_2C_6H_{11})COOH = C_{15}H_{26}O_6S_2$ (366.48):

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| calc.: | C | 49.16 | H | 7.15 | S | 17.50 % | equiv. | 366.5 |
| found: | » | 48.96 | » | 7.21 | » | 17.52 » | » | 364 |

Of this compound 0.55 g was heated in a bath to 150–170° for 10 minutes, after which no more carbon dioxide evolved. The residue was neutral. Recrystallisation from benzene-light petroleum yielded needles of m.p. 157–159°, unchanged on admixture with the 1,2-biscyclohexylsulphonylethane described in the following section.

### 3. 1,2-Biscyclohexylthioethane and 1,2-biscyclohexylsulphonylethane

A solution of sodium cyclohexyl mercaptide prepared from 7.9 g of cyclohexyl thiolacetate, 6 g of sodium hydroxide, 10 ml of water, and 50 ml of ethanol was treated with 4.7 g of 1,2-dibromoethane. Some heat was emitted and the solution soon became turbid and separated an oily bottom layer. The following day, much water was added and the mixture extracted with ether, the ether evaporated, and the residue dried at the pump on a water bath. Distilling at 0.2 mm gave 4.8 g of a main fraction of b.p. 135–137°, according to the analysis consisting of pure 1,2-biscyclohexylthioethane.

For $(C_6H_{11} \cdot S \cdot CH_2)_2 = C_{14}H_{26}S_2$ (258.47):

| | | | | | | |
|---|---|---|---|---|---|---|
| calc.: | C | 65.05 | H | 10.14 | S | 24.81 % |
| found: | » | 65.18 | » | 10.12 | » | 24.84 » |

$d_4^{20}$ 1.0248, $n_D^{20}$ 1.5383, mol.refr. 78.93, at. refr. of S 8.24.

Of the sulphide 1.3 g were dissolved in 20 ml of glacial acetic acid and oxidised with 5 ml of 30 % hydrogen peroxide. The initially turbid solution on shaking got clear with a slight emission of heat. The next day, most of the acetic acid was distilled off and the residue poured into 75 ml of water, precipitating 1.5 g of white crystals of m.p. 156–159°. Recrystallisation from 25 ml of benzene + 100 ml of light petroleum yielded aggregates of glistening needles, m.p. 156.5–159°, consisting of pure 1,2-biscyclohexylsulphonylethane.

For $(C_6H_{11} \cdot SO_2 \cdot CH_2)_2 = C_{14}H_{26}O_4S_2$ (322.47):

calc.: C 52.14 H 8.13 S 19.88 %
found: » 51.90 » 8.09 » 19.72 »

## III. 2-Cyclohexylthioacrylic acids

### 1. The higher-melting isomer, named A-acid

The propiolic acid used in the experiments was prepared by decarboxylation of the acid potassium salt of acetylenedicarboxylic acid in the way described by Perkin and Simonsen (20). As the partition coefficients of propiolic acid between ether and water was found to be as high as 2.59 (without correction for the dissociation), it was not found necessary to extract it 15 times as recommended in the original work. The acetylenedicarboxylic acid was prepared according to (21).

Some preliminary experiments were made to find the best reactional conditions for the addition of cyclohexanethiol to propiolic acid. At room temperature, without a solvent, or with small amounts of pyridine, or ethanolic sodium hydroxide, the reaction was too slow to be of any value. The same occurred if a mixture of the reactants was irradiated with ultraviolet light at room temperature, or if glacial acetic acid was used as a solvent. With excess of pyridine a rapid reaction led to black, tarry products. A rapid, exothermic reaction was started by heating the mixture, without a solvent, to about 70°. The temperature may not rise above 100°, otherwise the reaction runs out of control. A rapid reaction was also started by the addition of a small amount of conc. sulphuric acid.

A mixture of 7.0 g of propiolic acid and 11.6 g of cyclohexanethiol was heated in the way mentioned. After about one hour, the reaction was complete, the mixture being largely crystalline. Towards the end of the reaction, some carbon dioxide was evolved, especially when the temperature was high. The mixture was stirred with 25 ml of light petroleum, which left 8.9 g of crystal flakes undissolved, m.p. 149–151°. Recrystallisation from 50 ml of ethanol yielded 5.6 g of large, feather-like crystal aggregates of m.p. 151–154°.

For $C_6H_{11} \cdot S \cdot CH = CH \cdot COOH = C_9H_{14}O_2S$ (186.26):

calc.: C 58.03 H 7.58 S 17.21 % equiv. and mol. weight 186.3
found: » 58.04 » 7.61 » 17.19 » » 186.7 » » 187 (by the method of Rast (22))

The solution in light petroleum, obtained above, was neutralised with 39.8 ml of N-NaOH (phenolphthalein). The petroleum phase was freed from unreacted cyclohexanethiol by means of ethanolic mercuric chloride, the chloromercury cyclohexyl mercaptide formed (0.9 g) filtered off, the filtrate washed free from mercuric chloride with dilute hydrochloric acid, and finally evaporated, to yield

2.0 g of an oil, which in 40 ml of glacial acetic acid was oxidised with 7 ml of 30 % hydrogen peroxide. In two days there were formed 2.0 g of crystal needles of m.p. 184–186°, after recrystallisation from ethanol, of m.p. 187–189°. The product consisted of 1,1-biscyclohexylsulphonylethane, identical with the one described in section 3., p. 238.

For $(C_6H_{11} \cdot SO_2)_2CHCH_3 = C_{14}H_{26}O_4S_2$ (322.47):

| | | | |
|---|---|---|---|
| calc.: | C 52.14 | H 8.13 | S 19.88 % |
| found: | » 52.27 | » 8.27 | » 19.68 » |

Acidification of the neutral, aqueous phase with 10 ml of 5 N-HCl precipitated a red, sticky mass, that partly crystallised. The clear supernatant was extracted 4 times with ether, which was neutralised with 11.7 + 3.0 + 1.1 + + 0.5 = 16.3 ml of N-NaOH. Acidification of the aqueous solution, extraction with ether, drying the extract with calcium chloride, evaporation, and distilling the residue finally yielded 0.5 g of propiolic acid of equiv. 71.2, calc. 70.1.

The red, sticky mass was stirred with 50 ml of light petroleum (b.p. 40–60°), which left 1.7 g of palish red powder undissolved. The filtrate from this powder was neutralised with 12.2 ml of N-NaOH and the aqueous solution in the usual way oxidised with 90 ml of 0.95 N-$KMnO_4$. Acidification of the filtrate from the manganese dioxide slime gave 1.2 g of a somewhat sticky crystalline product, which after recrystallisation from benzene-light petroleum and ethanol-water had m.p. 171–172° and consisted of pure 2,2-biscyclohexylsulphonylpropionic acid.

For $(C_6H_{11} \cdot SO_2)_2CHCH_2COOH = C_{15}H_{26}O_6S_2$ (366.48):

| | | | | |
|---|---|---|---|---|
| calc.: | C 49.16 | H 7.15 | S 17.50 % | equiv. 366.5 |
| found: | » 49.01 | » 7.10 | » 17.36 » | » 370 |

The palish red powder (equiv. 190.0) undissolved in light petroleum (b.p. 40–60°), was recrystallised from 40 ml of light petroleum (b.p. 80–100°) to a yield of 0.35 g of m.p. (135)–143–148°, thus consisting of impure 2-cyclohexylthioacrylic acid. The mother-liquor was neutralised with 6.4 ml of N-NaOH. Acidification of the aqueous phase yielded 1.2 g product of m.p. 92–106°. Further recrystallisations from light petroleum did not alter the m.p., which was slightly depressed on admixture with a small amount of 2-cyclohexylthioacrylic acid of m.p. 151–154°. The product was separated in the way described in the next section, finally yielding an almost pure product of the lower-melting isomer, m.p. 111–113.5°.

When neutral or alkaline, aqueous solutions of the A-acid were heated for 4 hours in a water bath, almost no decomposition occurred, but the m.p.'s of the recovered acids were lowered to (125)–136–147°, indicating some isomerisation. Recrystallisation from ethanol gave the pure A-acid, m.p. 150–153°.

In acid solution the decomposition was rapid. A clear solution of 0.93 g of A-acid and 1.3 g of mercuric chloride in a mixture of 8 ml of n-propanol, 4 ml of 5 N-$H_2SO_4$, and 1 ml of water was heated on a water bath for one hour. The reaction vessel was connected with a receiver containing some water, via a condenser. Almost immediately glistening flakes began to form, and an odour of acetaldehyde was noticed. After completion of the reaction, the glistening flakes were sucked off, washed with water and acetone, yielding 1.6 g of chloromercury cyclohexyl mercaptide, equiv. 353.0, calc. 351.3. The acetone washings, on dilution with water, gave a slight opalescence but no precipitate. Ether only extracted minute amounts of acid material from the filtrate and aqueous washings. One third of the extracted solution was distilled off, taken together with the solution in the receiver, used during the reaction, and treated with a hot solution of 0.4 g of 2,4-dinitrophenylhydrazine in 20 ml of 5 N-HCl, to yield 0.36 g of hydrazone, after recrystallisation from methanol, of m.p. 163–165°, undepressed on admixture with authentic acetaldehyde 2,4-dinitrophenylhydrazone. SIMON and NEUBERG (19) give m.p. 164–165°.

If mercuric chloride and n-propanol were omitted, the same products resulted, but some A-acid was also recovered. In the unhomogeneous mixture the reaction proceeded much slower.

When a similar solution with mercuric chloride and n-propanol, but without sulphuric acid, was heated in the same way, the decomposition was much slower, a large amount of 2-cyclohexylthioacrylic acid being recovered. The m.p. of this was depressed. The same decomposition products were identified as described above.

A specimen of A-acid, 1.0 g, was melted and kept at 155° for half an hour. On being cooled down, the melt solidified, was crushed and stirred with 10 ml of light petroleum. The undissolved material, 0.62 g, had m.p. (91)–125–140° and equiv. 187.4. From the solution 0.14 g of a more impure product was recovered, m.p. 85–100°, equiv. 197.8.

The A-acid did not add cyclohexanesulphinic acid, at least not as smoothly as did the 1-cyclohexylthioacrylic acid. A clear solution was prepared from 0.93 g of A-acid, a solution of sodium cyclohexanesulphinate corresponding to 5 ml of N-NaOH, 2.5 ml of 2 N-HCl, and 5 ml of ethanol. Heating on a water bath for 3.5 hours yielded a turbid mixture, from which were isolated a small amount of cyclohexanethiol, cyclohexanesulphinic acid corresponding to 1.90 ml of N-NaOH, and 0.86 g of an acid of m.p. 90–100°, after recrystallisation from light petroleum 0.27 g of m.p. 98–106° and equiv. 192.4.

The partition coefficient of the A-acid between ether and water was determined as that of the 1-isomer and found to be 250.

### 2. The lower-melting isomer, named B-acid

The lower-melting 2-cyclohexylthioacrylic acid was the main mono-adduct in the reaction between cyclohexanethiol and propiolic acid catalysed by sulphuric acid. A mixture of 3.5 g of propiolic acid and 5.8 g of cyclohexanethiol was cooled to 5°. An exothermic reaction immediately started on addition of two drops of conc. sulphuric acid. By cooling with ice, the temperature was kept below 15° (mostly below 10°). After 2 hours the mixture was kept in an ice-safe for 5 days, then dissolved in 40 ml of ether, this neutralised with 53 ml of N-NaOH, the neutral ether on evaporation only giving a minute residue.

The neutral, aqueous solution was acidified with 11 ml of 5 N-HCl and extracted with 50 ml of ether. The ethereal solution was freed from propiolic acid by 8 extractions with 50 ml of water, which dissolved acids corresponding to 9.4 ml of N-NaOH. The ethereal solution was then neutralised with 36.7 ml of N-NaOH, the aqueous phase acidified and again extracted with ether, this finally being evaporated. The partly crystalline residue was stirred with light petroleum (b.p. 40–60°), 2.3 g of yellowish powder being undissolved, m.p. 90–109°. The solution was neutralised with 23 ml of N-NaOH, the neutral, aqueous solution then as usual oxidised with 190 ml of 0.95 N-$KMnO_4$. Acidification of the filtrate from the manganese dioxide slime precipitated a sticky mass, solidifying to a cake, weighing 3.1 g and of m.p. 145–155°. Recrystallisation from benzene-light petroleum and then from ethanol-water yielded 2.5 g of pure 2,2-biscyclohexylsulphonylpropionic acid of m.p. 169–171° and equiv. 366.2, calc. 366.5.

Recrystallisation of the palish yellow powder, undissolved in light petroleum (b.p. 40–60°), from 20 ml of light petroleum (b.p. 80–100°) afforded 1.9 g of faintly yellow aggregates of tablets of m.p. (98)–104–109° and equiv. 187.8. The m.p. was depressed by admixture of A-acid. The product gave a neutral solution with 10.00 ml of N-NaOH. The following extraction procedure was applied. The solution was diluted with 35 ml of water, treated with 2.50 ml of 2 N-HCl, and extracted with 50 ml of ether. The ethereal phase was extracted with 25 ml of 0.1 N-NaOH, the aqueous one acidified with 1.25 ml of 2 N-HCl and extracted with 50 ml of ether. The ethereal phase from the former extraction was finally neutralised with 25 ml of 0.1 N-NaOH, the corresponding aqueous phase equilibrated with the ethereal phase from the latter extraction, the aqueous phase of which was acidified with excess of acid, extracted with ether, and this neutralised with 25.5 ml of 0.1 N-NaOH. The original mixture was thus divided into three fractions, the first containing one fourth, enriched in the ethereal phase, the middle one containing one half in a two-phase solution, and the third containing one fourth, enriched in the aqueous phase. The procedure was repeated with the middle fraction, and with the middle fraction obtained from the foregoing one. Corresponding fractions were taken together, acidified, and the precipitates sucked off, finally yielding the following fractions:

1. Ether-enriched fraction, 0.80 g of white crystals, m.p. 107–110°.
2. Middle fraction, 0.23 g of white crystals, m.p. 104–110°.
3. Water-enriched fraction, 0.78 g of faintly yellow crystals of m.p. 100–108°.

The procedure is illustrated by fig. 1.

Recrystallisation of fraction 1 from 40 ml of light petroleum afforded 0.55 g of material of m.p. 109–113°, and one more recrystallisation gave material of m.p. 112–114°, which was considered to be the pure B-acid.

For $C_6H_{11} \cdot S \cdot CH = CH \cdot COOH = C_9H_{14}O_2S$ (186.26):

calc.: C 58.03 H 7.58 S 17.21 % equiv. and mol. weight 186.3
found: » 57.74 » 7.50 » 17.23 » » 186.5 » 194

Further quantities of the B-acid were obtained by repeating the systematic extractions and recrystallisations of the lower-melting fractions. Simultaneously the m.p. of the material in the aqueous phase was depressed, and in the ex-

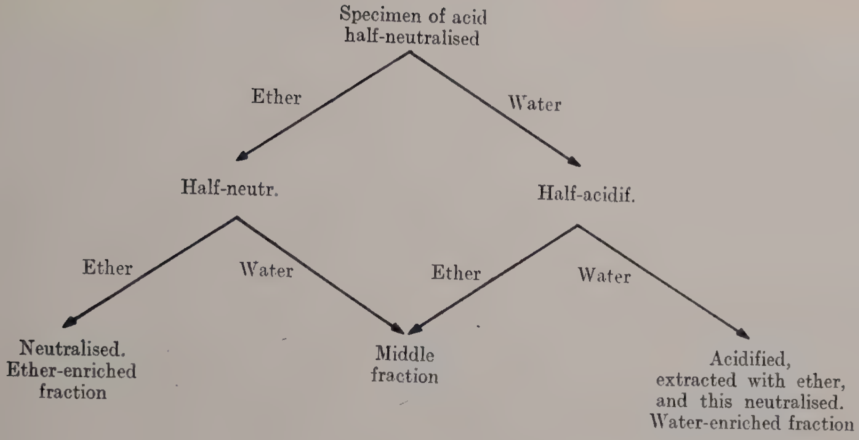

Specimen of acid half-neutralised
Ether
Water
Half-neutr.
Half-acidif.
Ether
Water
Ether
Water
Neutralised. Ether-enriched fraction
Middle fraction
Acidified, extracted with ether, and this neutralised. Water-enriched fraction

Fig. 1.

traction-procedure there was finally obtained 0.06 g of material of m.p. 93–96°, which on recrystallisation from 6 ml of light petroleum yielded a minute amount of material of m.p. 140–149°, mixed m.p. with A-acid 145–152°.

The melting point diagram of the system A-acid—B-acid is shown in fig. 2, and determined according to Rheinboldt (23). Weighed amounts of the acids were dissolved together in methanol and the mixture precipitated with water. See Table 3.

*Table 3*

| % A-acid | 100 | 75 | 50 | 33.6 | 25 | 0 |
|---|---|---|---|---|---|---|
| Thaw point (approx.) ..... | 151 | 100 | 92 | 92 | 92 | 112° |
| Melting point............ | 154 | 141 | 116 | 95.5 | 104.5 | 114° |

In acid or alkaline solution the B-acid behaved as the isomer. No decomposition was observed when an alkaline solution was heated for four hours in a water bath, but the m.p. of the recovered acid was lowered to (97)–102–108°. Recrystallisation from light petroleum afforded the pure B-acid, m.p. 112–114°.

The decomposition in acid solution was studied as in the case of the A-acid. From 0.3 g of B-acid, 0.5 g of mercuric chloride, 1.00 ml of 5.00 N-HCl, 1 ml of water, and 2 ml of n-propanol there was obtained 0.55 g of chloromercury cyclohexyl mercaptide, equiv. 349.8, and then 0.3 g of crude acetyldehyde 2,4-dinitrophenylhydrazone, m.p. 155–157°, after recrystallisation from methanol, m.p. 166–167°.

A specimen of B-acid was melted and kept at 155° for half an hour. After

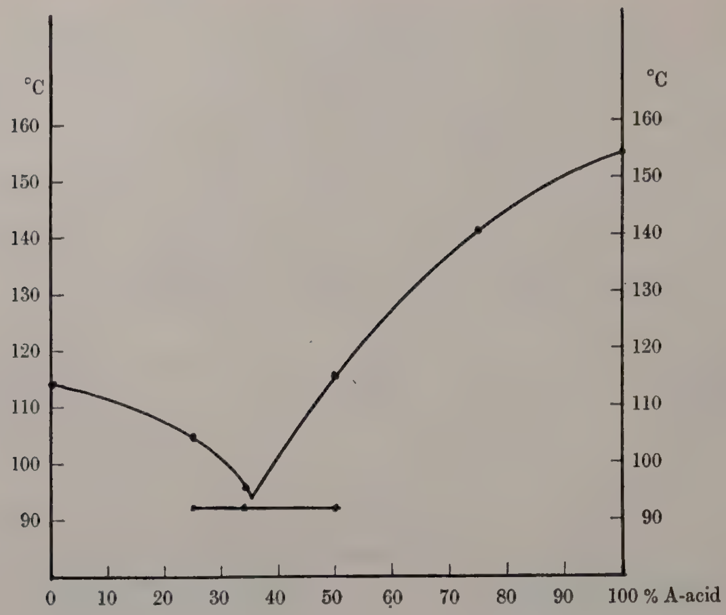


Fig. 2.

having been cooled down, the solidified melt was crushed and melted at 107–111°, after recrystallisation from light petroleum at 110–112°.

The partition coefficient of the B-acid was determined as described and found to be 750. This high value accounts for the fact, that the stronger B-acid was enriched in the ethereal phase during the extraction-procedure.

### 3. 1,1-Biscyclohexylsulphonylethane

A clear solution of 5.8 g of cyclohexanethiol, 2.2 g of pyruvic acid, 3 ml of conc. hydrochloric acid, and 15 ml of ethanol was heated for 6 hours on a water bath. Addition of much water precipitated an oil, which was taken up in ether. The ethereal solution was washed twice with water, then neutralised with 11.9 ml of N-NaOH. The neutral solution was then oxidised as usual with 90 ml of 0.95 N-$KMnO_4$. The filtrate from the manganese dioxide slime on acidification only precipitated minute amounts of sticky material, which was discarded. The manganese dioxide slime was exhaustively extracted with acetone, which dissolved 1.9 g of sticky crystals of m.p. 154–173°. Two recrystallisations from ethanol gave pure 1,1-biscyclohexylsulphonylethane of m.p. 187–189°.

For $(C_6H_{11} \cdot SO_2)_2CHCH_3 = C_{14}H_{26}O_4S_2$ (322.47):

| | | | |
|---|---|---|---|
| calc.: | C 52.14 | H 8.13 | S 19.88 % |
| found: | » 52.03 | » 8.13 | » 19.83 » |

## IV. Cyclohexylthiofumaric acid

Preliminary experiments showed that the reaction between cyclohexanethiol and acetylenedicarboxylic acid was slow when ethyl acetate or ether was used as a solvent. The same was true as regards the reaction between potassium acetylenedicarboxylate and cyclohexanethiol in ethanol-water. With pyridine as a solvent a rapid, exothermic reaction led to the formation of a compound, which, according to analysis, was an addition product of one molecule each of acetylenedicarboxylic acid, cyclohexanethiol, and pyridine. This interesting reaction was not further studied in this connection.

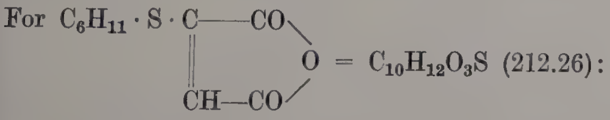
A satisfactory reaction occurred in glacial acetic acid. When 14.4 g of acetylenedicarboxylic acid (containing water of crystallisation, equiv. 72.0) and 11.6 g of cyclohexanethiol were dissolved in 50 ml of glacial acetic acid a slightly exothermic reaction was observed. The following day a yellow, crystalline crust had separated, and iodimetric titration of a sample of the reaction mixture showed that nearly 90 % of the thiol was consumed. After 3 days the yellow solution was decanted, the crust crushed, washed with glacial acetic acid, and sucked off, to yield 11.6 g of a product of m.p. 144–148° (efferv.), equiv. 114.9. Recrystallisation from ethyl acetate afforded pure cyclohexylthiofumaric acid as hard, pale-yellow crystals of m.p. 153–155° (efferv.). Boiling with activated charcoal in ethyl acetate did not decolourise the substance. The corresponding phenylthio- and phenyloxy-compounds, described by RUHEMANN and STAPLETON (5), were also yellowish.

For $C_6H_{11} \cdot S \cdot C(COOH) = CH \cdot COOH = C_{10}H_{14}O_4S$ (230.27):

calc.: C 52.16 H 6.13 S 13.92 equiv. 115.1
found: » 52.29 » 6.16 » 13.79 » 115.8

The decanted solution was freely evaporated and the residue dried on porous tile, yielding 11.2 g of yellowish powder, m.p. 117–140° (efferv.), equiv. 116.2. This was recrystallised from ethyl acetate, the mother-liquor freely evaporated, and the residue recrystallised in the same way. This procedure was repeated several times, and yielded fractions of cyclohexylthiofumaric acid of decreasing purity, as indicated by the progressively lowered m.p.'s. The final residue, 1.7 g, was recrystallised from 15 ml of benzene, to a yield of 0.46 g of a product of m.p. 133–137°. All these fractions, 7.0 g, were taken together and recrystallised from 25 ml of ethyl acetate, yielding 4.9 g of pure product of m.p. 151–153° (efferv.).

The benzene mother-liquor from the above-mentioned last fraction was neutralised with 15 ml of N-NaOH, and the aqueous solution acidified, to yield an oil, which slowly crystallised. After two weeks it was sucked off and yielded 1.0 g of crystal flakes of m.p. 75–77°. Recrystallisation from 15 ml of light petroleum gave featherlike crystals of m.p. 76–78°, according to the analysis consisting of cyclohexylthiomaleic anhydride.



$$\text{For } C_6H_{11} \cdot S \cdot \begin{array}{l} C\text{—}CO\diagdown \\ \| \qquad\qquad O \\ CH\text{—}CO\diagup \end{array} = C_{10}H_{12}O_3S \ (212.26):$$

calc.: C 56.58 H 5.70 S 15.10 % equiv. 106.1
found: » 56.79 » 5.85 » 15.09 » » 107.0 (in dioxane, sharp end point with phenolphthalein)

When neutral or alkaline solutions of sodium cyclohexylthiofumarate were heated in a water bath for 4 hours no decomposition was observed. The unchanged acid was recovered on acidification of the solutions.

In warm, aqueous medium with or without mineral acid the cyclohexylthiofumaric acid was decomposed. Preliminary experiments showed, that cyclohexanethiol, pyruvic acid, and carbon dioxide were formed, but there was also some indication of the formation of 1-cyclohexylthioacrylic acid. The corresponding reaction formulas are given in the introduction, p. 228.

In the case of reaction 4 a. there is formed as much carbon dioxide as cyclohexanethiol or less, in reaction 4 b. at most as much cyclohexanethiol as carbon dioxide. The experiments were therefore carried out in such a way, that the amounts formed of cyclohexanethiol and carbon dioxide could be determined. As cyclohexylthiofumaric acid is only slightly soluble in water, solutions were prepared with aqueous glycol, this alcohol being chosen on account of its high boiling point. The thiol was caught with mercuric chloride. A summary of the experiments is given in Table 4. A typical run was made as follows. Five millimoles (1.151 g) of cyclohexylthiofumaric acid were dissolved in 10 ($V_1$ in Table 4) ml of glycol, then 10 ($V_2$) ml of water and 1.4 g of mercuric chloride were added. The reaction vessel was fitted with a reflux condenser and carbon dioxide-free air aspirated through it, the gases were then dried with magnesium perchlorate and the carbon dioxide formed was finally absorbed in sodium hydroxide-asbestos. The solution was heated for one hour ($t$ min.), yielding 0.1004 g (2.28 ($m_1$) millimoles) of carbon dioxide and 0.5542 g (1.58 ($m_2$) millimoles) of chloromercury cyclohexyl mercaptide, washed with water and acetone. The filtrate and aqueous washings were diluted with water to about 70 ml. The turbid solution precipitated 0.05 g of crystal needles of m.p. 73–76° (turbid melt). The corresponding products from several experiments were taken together and purified by precipitating the filtered acetone solution with water, yielding 1-cyclohexylthioacrylic acid of m.p. 82–84° and equiv. 193. From this product the S-benzyl-*iso*-thiuronium salt was prepared, after recrystallisation from ethanol, of m.p. 164–165°, undepressed on admixture with authentic material. After addition of potassium bromide, the mother-liquor of the crude 1-cyclohexylthioacrylic acid was neutralised (in some experiments the acid was included) with 8.15 ml of N-NaOH ($v_1$). This figure is appreciably lower than that calculated from the yields of the products isolated (9.04 ml, $v_2$) possibly owing to formation of glycol esters. The neutral solution was freed from excess of mercuric chloride by treatment with 1.2 g of potassium xanthate. The filtrate from the precipitate formed was acidified and after one hour extracted twice with ether, the ethereal solutions being neutralised with $5.2 + 0.8 = 6.0$ ml of N-NaOH. The extracted, aqueous solution with 0.2 g of 2,4-dinitrophenylhydrazine in 30 ml of hot 5 N-HCl yielded 0.15 g of 2,4-dinitrophenylhydrazone of pyruvic acid, m.p. 216° (efferv.). Acidification of the neutral, aqueous solution of the extract gave an oil, that slowly and partly crystallised. Warm light petroleum extracted 0.1 g of cyclohexylthiomaleic anhydride of m.p. 75–78°, undepressed on admixture with authentic material. The residue was recrystallised from benzene to yield 0.1 g of unchanged cyclohexylthiofumaric acid of m.p. 151–152° (efferv.), equiv. 117.1. Free evaporation of the mother-liquor gave some sticky material, that did not crystallise.

From Table 4 may be seen that the reaction proceeded faster in the presence

*Table 4*

| $t$ | $V_1$ | $V_2$ | $v_1$ | $v_2$ | $m_1$ | $m_2$ | $m_1 - m_2$ |
|---|---|---|---|---|---|---|---|
| 30 | 10 | 10 | 8.30 | 8.77 | 1.81 | 1.08 | 0.73 |
| 60 | 10 | 10 | 8.15 | 9.04 | 2.28 | 1.58 | 0.70 |
| 120 | 10 | 10 | 8.25 | 9.53 | 2.74 | 2.27 | 0.47 |
| 60 | 5 | 15 | 8.85 | 9.14 | 2.74 | 1.88 | 0.86 |
| 60 | 5 | 15[1] | 29.40 | 29.91 | 4.32 | 4.23 | 0.09 |
| 60 | 5 | 15[2] | 4.00 | | 4.46 | $<3.5$[3] | |
| 75 | 10[4] | 10 | 9.60 | 9.43 | 0.79 | 0.22 | 0.57 |

[1] 10.00 ml of 2 N-HCl + 5 ml of water. [2] 5.00 ml of N-NaOH + 10 ml of water. [3] Heavily contaminated with calomel. [4] Dioxane.

of excess of mineral acid, that the decarboxylation reaction was enhanced in a solution originally partly neutralised, and that dioxane instead of glycol retarded the reaction. That the reaction proceeded via an intermediate or by several routes is also obvious.

A clear solution, prepared from 1.15 g of cyclohexylthiofumaric acid, a solution of sodium cyclohexanesulphinate corresponding to 5 ml of N-NaOH, 2.5 ml of 2 N-HCl, and 5 ml of ethanol, was heated with reflux on a water bath for 3.5 hours. When cold, the mixture, which had deposited some oil, was extracted with ether, this being neutralised with 9.85 ml of N-NaOH. The neutral, ethereal solution contained a minute amount of cyclohexanethiol. Acidification of the neutral, aqueous solution precipitated an oil, which was filtered off. The filtrate was extracted twice with benzene, the benzene phase neutralised with 1.10 + 0.20 = 1.30 ml of N-NaOH, and the aqueous solution obtained precipitated with a solution of 0.3 g of S-Benzyl-*iso*-thiuronium chloride in 15 ml of water. The precipitate formed, 0.15 g, was finally purified by precipitation of its filtered solution in methanol with acetone. Pure S-benzyl-*iso*-thiuronium cyclohexanesulphinate of m.p. and mixed m.p. 157–159° was obtained. The oil mentioned above was taken up in ether, the solution was neutralised with 6.4 ml of N-NaOH, the oil reprecipitated, dried in vacuo, and extracted with several portions of boiling light petroleum which dissolved some of it and on cooling deposited 0.3 g of crude 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid, after recrystallisation from the same solvent of m.p. and mixed m.p. 113–115°. The yellow, undissolved, sticky residue on recrystallisation from benzene first gave a fraction of impure cyclohexylthiofumaric acid of m.p. 147–149° (efferv.) and equiv. 139.4, and then a minute amount of colourless crystal powder of m.p. 174–175° (efferv.) and equiv. 189, for a cyclohexylthio-cyclohexylsulphonylsuccinic acid calc. 189.2.

The thermal decomposition of cyclohexylthiofumaric acid was complicated. In an experiment, 11.5 g of the acid were melted and kept at 160° for 15 minutes. After that time there was practically no more evolution of carbon dioxide. The yellow-red oily residue weighed 9.4 g, corresponding to the splitting off of exactly one carboxyl group. It was dissolved in 50 ml of ether, and the solution washed with three 30 ml portions of water, which were neutralised with 2.20 + 1.00 + 0.60 = 3.80 ml of N-NaOH and then with 2,4-dinitrophenylhydrazine gave the corresponding hydrazone of pyruvic acid, after recrystallisation from methanol, of m.p. 216° (efferv.). The ethereal solution was then neutralised (unsharp end point) with 50 ml of N-NaOH. The neutral, ethereal solution with ethanolic mercuric chloride gave 0.26 g of chloromercury cyclohexyl mercaptide of equiv. 351.5. Acidification of the neutral, aqueous solution precipitated an oil, which was taken up in 30 ml of benzene. The benzene solution was dried with calcium chloride and then treated with 70 ml of light petroleum, which overnight precipitated a brownish crystal crust, weighing 0.9 g and of m.p. 126–129°. Further 80 ml of light petroleum yielded another portion, weighing 2.1 g and with the same m.p. Two recrystallisations from 50 % aqueous methanol afforded white crystal needles of m.p. (130)–133–135°.

For $(C_6H_{11}\cdot S\cdot CH = CHCOOH)_2 = C_{18}H_{28}O_4S_2$ (372.52):

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| calc.: | C | 58.03 | H | 7.58 | S | 17.21 % | equiv. | 186.3 | mol. w. | 372.5 |
| found: | » | 58.04 | » | 7.63 | » | 17.18 » | » | 187.3 | » » | 360 |

The benzene-petroleum mother-liquor of the crude product, on free evaporation yielded an oil which slowly hardened with signs of crystallisation, but the crystals could not be freed from the sticky residue, which was not absorbed in porous tile. It was therefore not further examined.

The crystalline product obtained, henceforth named the dimer, was subjected to some experiments. When a neutral solution of the sodium salt was heated as usual, no decomposition was observed and the dimer recovered almost unchanged. When a clear solution of 0.19 g of dimer, 0.3 g of mercuric chloride, 2 ml of n-propanol, 2 ml of water, and 1 ml of 5 N-HCl was heated, with reflux, for 4 hours on a water bath, some oil was slowly precipitated, but practically no solid material. The oil could not be brought into crystalline form, and was not further investigated.

A neutral solution prepared from 0.57 g of the dimer and 3.15 ml of N-NaOH was treated with a further 3.15 ml of the alkali and then heated for 4 hours on a water bath. When cold, it was neutralised with 1.55 ml of 2 N-HCl, and the turbid solution, with an odour of cyclohexanethiol, was extracted with ether. The ethereal solution with ethanolic mercuric chloride afforded 0.35 g of chloromercury cyclohexyl mercaptide of equiv. 352.0. Acidification of the aqueous phase precipitated 0.42 g of yellow, microcrystalline powder of m.p. (175)–190–213°. Recrystallisation from 7 ml of methanol + 5 ml of water yielded 0.15 g of yellow, fine needles of m.p. 218–222°. A neutral solution of the sodium salt was colourless. The product was analysed.

For $C_6H_{11}\cdot S\cdot C_4H_3(COOH)_2 = C_{12}H_{16}O_4S$ (256.31);

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| calc.: | C | 56.23 | H | 6.29 | S | 12.51 % | equiv. | 128.2 |
| found: | » | 56.13 | » | 6.23 | » | 12.48 » | » | 129.9 |

An attempt was also made to add cyclohexanesulphinic acid to the dimer. The experiment was carried out as the corresponding one with 2-cyclohexylthioacrylic acid (A-acid, p. 235), using half quantities. No addition product was isolated, only parts of the original reactants.

## V. Cyclohexylthiomaleic anhydride

The preparation of cyclohexylthiomaleic anhydride was described in the foregoing section, p. 239. It was more conveniently obtained from the evaporation residue mentioned there by extraction with boiling light petroleum. On being cooled down the solution first deposited some cyclohexylthiofumaric acid. The supersaturated solution after decantation then rapidly deposited almost pure, colourless crystals of cyclohexylthiomaleic anhydride. When the anhydride was melted and heated no evolution of gas was observed below 215°.

When neutral or alkaline solutions of sodium cyclohexylthiomaleate were heated in a water bath for 4 hours, no decomposition was observed. Acidification of the cold solution precipitated an oil, that was taken up in ether. Free evaporation overnight gave crystals of almost pure cyclohexylthiomaleic anhydride, m.p. 73–87°, after recrystallisation from light petroleum m.p. 75–77°. The high final melting temperature of the crude product may indicate some isomerisation to the fumaric acid derivative.

A solution of sodium cyclohexylthiomaleate, corresponding to 5.00 ml of N-NaOH, was mixed with 2.5 ml of glycol, 0.7 g of mercuric chloride, 1.5 ml of water, and 1 ml of 5 N-HCl. The clear solution so obtained was heated for one hour on a water bath in the way described for the fumaric acid derivative. There was obtained 0.1025 g (2.33 millimoles) of carbon dioxide and 0.6241 g (1.78 millimoles) of chloromercury cyclohexyl mercaptide. The filtrate from the latter was treated with potassium bromide, neutralised with 4.32 (calc. 4.55) ml of N-NaOH, and freed from mercuric chloride with potassium xanthate. After acidification, ether extracted acids corresponding to 1.25 ml of N-NaOH. From these a minute amount of cyclohexylthiomaleic anhydride was finally obtained. The extracted solution with 2,4-dinitrophenylhydrazine gave a precipitate of the corresponding hydrazone of pyruvic acid, m.p. 212–213° (efferv.).

The reaction between cyclohexylthiomaleic acid and cyclohexanesulphinic acid was investigated in much the same way as the corresponding reaction with the fumaric acid derivative, but on account of scarcity of material only with one fifth of the quantities. A small amount of cyclohexanesulphinic acid was recovered and identified as the S-benzyl-*iso*-thiuronium salt. There was obtained some crude 1-cyclohexylthio-2-cyclohexylsulphonylpropionic acid of m.p. 107–113°, not depressed on admixture with authentic material. Finally a minute amount of an acid of m.p. 180–182° (efferv.) and equiv. 175 was isolated. The mixed m.p. with the corresponding product from cyclohexylthiofumaric acid, of m.p. 174–175° (efferv.), was 167–168° (efferv.). Thus it might have been an isomeric cyclohexylthio-cyclohexylsulphonylsuccinic acid, equiv. calc. 189.2.

## VI. Ionisation constants and solubility products

### 1. Experimental procedure

As all the unsaturated cyclohexylthio-carboxylic acids prepared were slightly soluble in water, the method of Back and Steenberg (11) was used. Two milliequivalents of the acid to be investigated were dissolved, with exclusion of carbon dioxide, in a slight excess of 0.1 N-NaOH (about 20.5 ml) and the solution made up to 400 ml with boiled, distilled water. This solution was titrated with 0.1 N-HCl, magnetic stirring being applied throughout the determination. The pH was continuously measured with a "pH Meter 22" from Radiometer, with the use of a shielded glass electrode ("G 202") and a saturated calomel electrode ("K 201"). No precautions were made to keep the temperature constant, as the variations in the temperature (20°) of the laboratory during the measurements were less than one degree. The pH scale of the instrument was checked before and after each measurement, the pH standards recommended by GUGGENHEIM and SCHINDLER (24) being used. No correction was made for diffusion potentials.

## 2. Method of calculation

Though the determinations were not made with the highest possible precision it was still necessary to use activities instead of concentrations in the calculation of the ionisation constants, because the dibasic acids were rather strong. The activity of the hydrogen ion was taken from KIELLAND (25) and those of the other ions were estimated by means of the well known DEBYE-HÜCKEL formula

$$-\log f_i = 0.5\, i^2 \sqrt{\mu} / (1 + \sqrt{\mu}) \quad \text{(for symbols see below)}$$

SÖDERBÄCK (16) extensively discussed the calculation of ionisation constants from titration data. His formulas were derived for the cases where acids are titrated with alkali, and must be slightly modified for the actual case of titration of sodium salts with strong acid. The following symbols are used:

$C$ total concentration of acid to be measured, $H_2A$
$C_x$ concentration of molecular species $x$
$a_x$ activity » » » »
$f_i$ activity coeff. of ion of charge $i$
$K_i$ thermodynamical ionisation constant of ionisation stage $i$
$Me^+$ cation of alkali used
$X^-$ anion of acid used for the titration
$\mu$ ionic strength

Using the equilibrium conditions, the condition of electroneutrality, and stoichiometric relations the following titration equation is derived (compare 16):

$$1. \qquad K_1 K_2 (B-2)/f_2 + K_1 a_H + (B-1)/f_1 + B a_{H^+}^2 = 0$$

$B$ is defined by the relation:

$$2. \qquad B \cdot C = C_{Me^+} + C_{H^+} - C_{X^-} - C_{OH^-} = C_{HA^-} + 2\,C_{A^=}.$$

For the ionic strength the following relation is obtained by means of the foregoing equation:

$$3. \qquad \mu = C_{Me^+} + C_{H^+} + C_{A^=}.$$

With the low concentrations used in the experiments the variation of the activity coefficients lies within the experimental error of the pH values, estimated to be 0.02 pH unit. One finds:

in the case of the monobasic acids $\log f_1 = \log f_{H^+} = -0.03$
» » » » » dibasic acids $\log f_1 = -0.04$, $\log f_2 = -0.15$, $\log f_{H^+} = -0.03$.

Rearrangement of equation 1. leads to the following logarithmic expressions for the p$K$ values:

4 a. $$\mathrm{p}K_1 = \mathrm{pH} - \log \frac{B}{1-B} + \log (1+\alpha) - \log f_1 \qquad (B < 1)$$

4 b. $$\alpha = \frac{K_2 (2-B) f_1}{a_{H^+} (1-B) f_2}$$

5 a. $$\mathrm{p}K_2 = \mathrm{pH} - \log \frac{B-1}{2-B} - \log (1+\beta) + \log f_1 - \log f_2 \qquad (B > 1)$$

5 b. $$\beta = \frac{a_{H^+} \cdot B \cdot f_1}{K_1 (B-1)}.$$

The correction terms $\alpha$ and $\beta$ are small if the quotient $K_1/K_2 = q$ is large and $B$ not approaching 1. By omitting the correction terms it is therefore possible to calculate approximative values of the ionisation constants, which then may be used for the accurate calculation. In this way a value for the constant is obtained for each point of the part of the titration curve, that corresponds to the constant. By putting $K_2 = 0$ (and $\alpha = 0$) equation 4 a. is applicable also to the monobasic acids, and corresponds to the one used by BACK and STEENBERG (11).

The solubility products of the dibasic acids were too large to be reached during the titrations. As the cyclohexylthiomaleic acid did not crystallise, they were not considered to be of much value, thus no determination was made with higher concentrations. The solubility products ($K_s$) and solubilities ($s$) of the monobasic acids were determined according to (11):

6 a. $$\mathrm{p}K_s = \mathrm{pH} - \log C \cdot B - \log f_1$$

6 b. $$s = K_s / K \quad (s = \text{solubility of undissociated acid}).$$

The accuracy of the determinations was estimated by formation of the differentials d$\mathrm{p}K_1$ and d$\mathrm{p}K_2$ (and d$\mathrm{p}K_s$). The exact, rather complicated expressions may be derived by differentiation of the titration equation 1. As may be expected, the simpler relations calculated from 4 a. and 5 a. (and 6 a.), neglecting the correction terms, give reliable results. It is found, that in those parts of the titration curve, where $B$, $|B-1|$, and $|B-2|$ are not very small, only the errors of the pH determination, estimated to 0.02 pH unit, are of importance. Then the following relations result:

7. $$\mathrm{dp}K_1 = \left(1 + \frac{K_1}{C \cdot B^2}\right) \mathrm{dpH}$$

8. $$\mathrm{dp}K_2 = \left(1 + \frac{K_2}{C(B - 1)^2}\right) \mathrm{dpH}$$

9. $$\mathrm{dp}K_s = \left(1 + \frac{K_s}{C^2 B^2}\right) \mathrm{dpH}$$

## 3. Results

The complete experimental data of the titrations of A-acid and cyclohexylthiofumaric acid are given in Tables 5 and 6. For the other acids the final results only are reproduced, in Table 7, which also contains results obtained with acetic and fumaric acid, titrated for comparison. The agreement with the values reported in the literature seems satisfactory.

**2-Cyclohexylthioacrylic acid, A-acid.** Two millimoles (0.3725 g) were dissolved in 20.49 ml of 0.1004 N-NaOH. The hydrochloric acid used for the titration was 0.1007 N. From the titration curve the equivalence point (neutral salt) was determined to 0.52 ml. When 6.53 ml of acid had been added, the solution was supersaturated, and flocculation soon began with rise of pH. The values obtained in the extremities of the curve are put in parantheses and not used (see above). Simple means are calculated from the other values, which on the whole were very constant.

*Table 5*

| ml HCl | pH | p$K'$ | ml HCl | pH | p$K'$ | p$K'_s$ |
|---|---|---|---|---|---|---|
| 0.00 | 9.83 | | 4.53 | 5.50 | 4.90 | |
| 0.10 | 9.70 | | 5.54 | 5.38 | 4.91 | |
| 0.16 | 9.64 | | 6.53 | 5.31 | 4.94 | |
| 0.26 | 9.46 | | 6.53 | 5.82 | | 8.27 |
| 0.37 | 9.17 | | 8.55 | 5.73 | | 8.26 |
| 0.47 | 8.66 | | 10.60 | 5.63 | | 8.25 |
| 0.56 | 7.51 | (4.81) | 12.60 | 5.52 | | 8.24 |
| 0.66 | 7.08 | (4.93) | 14.57 | 5.40 | | 8.25 |
| 0.80 | 6.80 | (4.97) | 16.60 | 5.21 | | 8.24 |
| 0.90 | 6.68 | (4.98) | 18.56 | 4.91 | | 8.25 |
| 1.00 | 6.58 | (4.98) | 19.55 | 4.61 | | 8.24 |
| 1.32 | 6.31 | 4.93 | 20.56 | 4.07 | | (8.30) |
| 1.53 | 6.20 | 4.93 | 21.55 | 3.57 | | (8.35) |
| 2.52 | 5.87 | 4.92 | 22.54 | 3.31 | | (7.89) |
| 3.51 | 5.65 | 4.90 | 23.55 | 3.14 | | (7.54) |

The constants in the table are "incomplete" ($= \mathrm{p}K + \log f_1$). The mean values are p$K' = 4.92$, p$K'_s = 8.25$.

**Cyclohexylthiofumaric acid.** One millimole (0.2303 g) of the acid was dissolved in 20.45 ml of 0.1004 N-NaOH. The equivalence point (neutral salt) was at 0.50 ml.

*Table 6*

| ml HCl | pH | $pK_2'$ (uncorr.) | $pK_2'$ (corr.) | ml HCl | pH | $pK_2'$ (uncorr.) | $pK_2'$ (corr.) |
|---|---|---|---|---|---|---|---|
| 0.00 | 9.92 | | | 10.34 | 3.87 | 4.99 | (4.93) |
| 0.10 | 9.77 | | | 10.45 | 3.84 | 5.01 | (4.94) |
| 0.20 | 9.62 | | | 10.53 | 3.82 | 5.03 | (4.95) |
| 0.29 | 9.45 | | | 10.70 | 3.77 | 5.06 | (4.96) |
| 0.44 | 8.68 | | | 10.80 | 3.74 | 5.09 | (4.97) |
| 0.50 | 8.05 | | | 11.00 | 3.69 | 5.16 | (4.99) |
| 0.63 | 6.86 | 4.98 | (4.98) | 11.51 | 3.56 | 5.44 | (5.03) |
| 0.73 | 6.60 | 4.99 | (4.99) | 12.03 | 3.44 | 5.96 | (4.96) |
| 0.87 | 6.39 | 4.99 | (4.99) | 12.50 | 3.36 | | |
| 1.04 | 6.20 | 4.96 | (4.96) | 13.00 | 3.27 | $pK_1'$ | $pK_1'$ |
| 1.51 | 5.84 | 4.88 | 4.88 | 13.50 | 3.22 | 1.82 | (2.02) |
| 2.05 | 5.62 | 4.88 | 4.88 | 14.00 | 3.14 | 1.69 | 1.88 |
| 2.52 | 5.49 | 4.89 | 4.89 | 14.56 | 3.08 | 1.72 | 1.86 |
| 3.01 | 5.37 | 4.89 | 4.89 | 15.00 | 3.04 | 1.78 | 1.89 |
| 3.53 | 5.25 | 4.89 | 4.89 | 15.51 | 2.99 | 1.74 | 1.84 |
| 4.02 | 5.15 | 4.88 | 4.88 | 16.00 | 2.95 | 1.74 | 1.82 |
| 4.53 | 5.06 | 4.89 | 4.89 | 16.50 | 2.92 | 1.82 | 1.88 |
| 5.00 | 4.98 | 4.89 | 4.89 | 17.00 | 2.87 | 1.67 | 1.74 |
| 5.53 | 4.89 | 4.89 | 4.89 | 17.53 | 2.85 | 1.80 | 1.85 |
| 6.00 | 4.81 | 4.89 | 4.89 | 17.99 | 2.83 | 1.79 | 1.84 |
| 6.54 | 4.71 | 4.88 | 4.88 | 18.53 | 2.79 | 1.78 | 1.82 |
| 7.00 | 4.63 | 4.89 | 4.89 | 19.03 | 2.76 | 1.74 | 1.78 |
| 7.50 | 4.53 | 4.88 | 4.88 | 19.50 | 2.75 | 1.88 | 1.91 |
| 8.02 | 4.43 | 4.89 | 4.89 | 20.00 | 2.73 | 1.91 | 1.94 |
| 8.53 | 4.33 | 4.90 | 4.89 | 20.50 | 2.70 | 1.84 | 1.87 |
| 9.00 | 4.22 | 4.90 | 4.89 | 21.00 | 2.67 | 1.75 | 1.78 |
| 9.50 | 4.10 | 4.93 | 4.91 | 22.00 | 2.64 | 1.85 | 1.87 |
| 9.72 | 4.03 | 4.92 | 4.89 | | | | |
| 9.82 | 3.98 | 4.89 | 4.86 | | | | |
| 10.10 | 3.94 | 4.97 | 4.92 | | | | |

The mean values are $pK_1' = 1.85$, $pK_2' = 4.89$ ("incomplete" constants).

The values for cyclohexylthiomaleic acid were equally constant. Thus no formation of anhydride occurred during the titration.

*Table 7*

| Acid | $pK$ | $pK_s$ | $s \times 10^4$ |
|---|---|---|---|
| 1-Cyclohexylthioacrylic acid . . . . . . . . . . . . . . . . . . . . . . | $3.40 \pm 0.02$ | $5.99 \pm 0.03$ | 25.9 |
| 2- » A-acid . . . . . . . . . . . . . . . . . . . | $4.95 \pm 0.02$ | $8.25 \pm 0.02$ | 4.68 |
| 2- » B- » . . . . . . . . . . . . . . . . . . . | $4.70 \pm 0.02$ | $8.23 \pm 0.02$ | 2.95 |
| Acetic acid . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | $4.76 \pm 0.02$ | | |
| | $pK_1$ | $pK_2$ | |
| Cyclohexylthiofumaric acid . . . . . . . . . . . . . . . . . . . . . | $1.89 \pm 0.15$ | $5.00 \pm 0.02$ | |
| Cyclohexylthiomaleic acid . . . . . . . . . . . . . . . . . . . . . . | $1.90 \pm 0.15$ | $5.93 \pm 0.02$ | |
| Fumaric acid . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | $3.03 \pm 0.04$ | $4.47 \pm 0.02$ | |

For acetic acid HARNED and EHLERS (26) give $pK$ 4.76, and for fumaric acid ASHTON and PARTINGTON (13) give $pK_1$ 3.02 and $pK_2$ 4.32.

REFERENCES. 1. **B. Weibull:** Ark. Kemi *3*, no. 23 (1951). — 2. **R. Escales** and **E. Baumann:** Ber. *19*, 1787 (1886). — 3. **W. Autenrieth:** Ann. *254*, 222 (1889). — 4. **A. W. Johnson:** The Chemistry of the Acetylenic Compounds. II. The Acetylenic Acids. London 1950. — 5. **S. Ruhemann** and **H. E. Stapleton:** J. Chem. Soc. *77*, 1179 (1900). — 6. **B. R. Baker** and **M. V. Querry:** J. Org. Chem. *15*, 417 (1950). — 7. **L. N. Owen** and **M. U. S. Sultanbawa:** J. Chem. Soc. *1949*, 3109. — 8. **R. Escales** and **E. Baumann:** Ber. *19*, 2814 (1886). — 9. **A. Wohl** and **W. Emmerich:** Ber. *33*, 2760 (1900). — 10. **L.-J. Simon:** C. R. Acad. Sci., Paris *137*, 855 (1903). — 11. **E. Back** and **B. Steenberg:** Acta Chem. Scand. *4*, 810 (1950). — 12. **Ch. Moureu** and **A. Boutaric:** J. chim. phys. *18*, 348 (1920). — 13. **H. W. Ashton** and **J. R. Partington:** Trans. Faraday Soc. *30*, 598 (1934). — 14. **E. Larsson:** Ber. *63*, 1348 (1930). — 15. **J. M. Lovén:** Z. physik. Chem. *13*, 550 (1894). — 16. **E. Söderbäck:** Ark. Kemi Min. Geol. *11 A*, no. 8 (1933). — 17. **A. D. Mitchell:** British Chemical Nomenclature. London 1948. — 18. **B. Weibull:** Ark. Kemi Min. Geol. 23 *A*, no. 18 (1946). — 19. **E. Simon** and **C. Neuberg:** Biochem. Z. *232*, 479 (1931). — 20. **W. H. Perkin**, jun., and **J. L. Simonsen:** J. Chem. Soc. *91*, 834 (1907). — 21. Organic Syntheses, Coll. Vol. II, p. 10. — 22. **K. Rast:** Ber. *55*, 1051 (1922). — 23. **H. Rheinboldt:** J. prakt. Chem. [2] *111*, 242 (1925). — 24. **E. A. Guggenheim** and **T. D. Schindler:** J. Phys. Chem. *38*, 533 (1934). — 25. **J. Kielland:** J. Amer. Chem. Soc. *59*, 1675 (1937). — 26. **H. S. Harned** and **R. W. Ehlers:** J. Amer. Chem. Soc. *55*, 652 (1933).

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# Optically active higher aliphatic compounds. IX

## The synthesis of the dextrorotatory diastereoisomers of 3,4-dimethyldocosanoic acid

By STINA STÄLLBERG-STENHAGEN

With 1 figure in the text

It has been shown previously (Parts V (1) and VII (2)) that the optical rotation of long chain compounds with two active centres separated by at least two methylene groups can be calculated with a fair degree of accuracy by adding the contributions of the separate centres, using data obtained from monosubstituted compounds with the corresponding active centre in similar position along the chain.

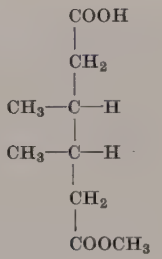
As was pointed out in Part VII (2) it is not to be expected that the simple addition rule will hold when the active centres are adjacent to each other. In order to study this question, a method of introducing two optically active centres in α-position to each other, with carbon atoms carrying methyl side chains, has been developed, using resolved half-esters of DL- and *meso*-3,4-dimethyladipic acid respectively as initial material. The method is analogous to the method of introducing a single optically active centre with a carbon atom carrying a methyl side chain described in Parts II (3) and VI (4) of this series, which uses (+)- and (—)-methyl hydrogen β-methylglutarate as initial material.

The preparation of the diastereoisomeric 3,4-dimethyladipic acids has been described by FALTIS and WAGNER (5). Treatment of β-iodobutyric acid with copper powder at elevated temperatures gave a mixture of acids, from which the higher melting acid of m. p. 133° was readily obtained pure by crystallization. For the lower melting acid, which was much more difficult to obtain, a melting point of 104–105° is recorded. A mixture of DL- and *meso*-3,4-dimethyladipic acid was later prepared by VOGEL (6) by decarboxylation of 2,3-dimethylbutane-1,1,4,4-tetracarboxylic acid. The ester of the tetracarboxylic acid was prepared by reductive coupling of ethyl ethylidenemalonate by moist aluminium amalgam.

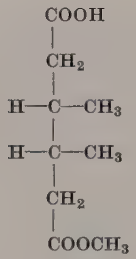
In the course of the present work relatively large quantities of the 3,4-dimethylsubstituted adipic acids were needed, and it was found that both the above routes may be used for preparations on a fairly large scale. The method of FALTIS and WAGNER gives, as was pointed out by these authors, the higher and the lower melting acid in about equal proportions. The method of VOGEL, on the other hand, gives a much higher proportion of the higher melting acid, evidently because, during the decarboxylation of the tetracarboxylic acids, a large part of the lower melting 3,4-dimethyladipic acid is further transformed by decarboxylation and ring closure into 3,4-dimethylcyclopentanone-1. As a result, the method of VOGEL is very convenient when the higher melting acid alone is required. VOGEL (6) has prepared the higher melting tetracarboxylic acid in a pure form (m. p. 185–185.5°) and this acid was provisionally regarded as the racemic form. In the course of the present work, it was found that the higher melting tetracarboxylic acid on decarboxylation gives the higher melting 3,4-dimethyladipic acid. As will be shown below the latter is the *meso*-acid, and the higher melting tetracarboxylic acid is therefore the *meso*-form, and not the racemic form as supposed by VOGEL.

The melting point of the *meso*-acid obtained in the present work was 133–134°, in close agreement with the figure given by FALTIS and WAGNER.

A pure specimen of *meso*-3,4-dimethyladipic acid was converted into the monomethyl ester by partial esterification. The half-ester thus obtained consists of equal amounts of the enantiomorphs I a and I b shown below.



I a



I b

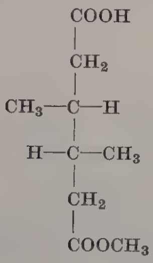
The laevorotatory enantiomorph, $[\alpha]_D^{25} -1.36°$ (homogeneous) was readily obtained by crystallization of the cinchonidine salt from 60 % acetone. A constant rotation was usually reached after three crystallizations. Hydrolysis of the active half-ester gave a completely inactive dibasic acid, thus indicating that the higher melting 3,4-dimethyladipic acid is the *meso*-form.

The attempts to obtain the DL-acid in a pure form by direct crystallization did not succeed. Fractions melting at about 105° with a rather small melting range could be obtained, but further crystallization showed that these fractions were mixtures, and it appeared doubtful that FALTIS and WAGNER had obtained the DL-acid in a pure state.

It was found in the earlier attempts to resolve the inactive half-ester of the *meso*-acid that, if the sample used for the preparation of the half-ester contained a small amount of the DL-acid (indicated by a melting point lowered by two to three degrees), it was impossible to reach the optical rotation recorded above even after five crystallizations. A specimen of active half-ester obtained in a run when a slightly impure *meso*-acid had been used as initial material was esterified to the dimethyl ester. After distillation, the latter showed a small dextrorotation. The above findings suggested that the cinchonidine salts of the enantiomorphic pair of half-esters derived from the DL-3,4-dimethyladipic acid are considerably less soluble than those of the corresponding pair derived from the *meso*-acid. This indicated that the enantiomorphic half-esters of the *meso*-acid could be removed from those of the DL-acid by recrystallization of the cinchonidine salts of the mixed methyl half-esters.

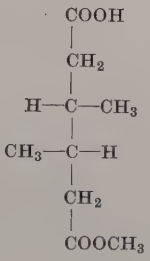
However, the DL-acid cannot be obtained directly in this manner, as the acid obtained by hydrolysis of the half-esters purified via the cinchonidine salts is more or less resolved. A pure specimen of DL-3,4-dimethyladipic acid was therefore prepared by mixing the appropriate amounts of partially resolved (+)- and (—)-half-ester, hydrolyzing and recrystallizing from water. The DL-acid obtained in this manner melted att 115.5–116.6°. The same m. p. was also found in previously melted material that had been cooled to room temperature. On reheating immediately after solidification of the melt, remelting occurred at 107–108°. The acid in thus dimorphous, but the lower melting modification is stable only near the m. p.

Repeated crystallization of the cinchonidine salt of the half-ester of the DL-acid, prepared by partial esterification of the acid or by partial hydrolysis of the dimethyl ester, finally gave the pure salt of the dextrorotatory enantiomorph. The steric connection between the latter and glyceraldehyde is not yet known, and it is therefore not possible to say whether the dextrorotatory enantiomorph is correctly represented by formula II a or by II b.



$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CH_3—C—H \\ | \\ H—C—CH_3 \\ | \\ CH_2 \\ | \\ COOCH_3 \end{array}$$

II a



$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ H—C—CH_3 \\ | \\ CH_3—C—H \\ | \\ CH_2 \\ | \\ COOCH_3 \end{array}$$

II b

The resolution proceeds slowly, and at least 12 recrystallizations were necessary to reach a constant rotation. The course of a typical resolution experiment is shown in Fig. 1. The loss in each recrystallization is rather small, however, and the yield of pure enantiomorph is around 30 % of the theoretical.

Hydrolysis of the dextrorotatory enantiomorph of $[\alpha]_D^{25} + 4.91°$ (homogeneous) gave the *laevorotatory 3,4-dimethyladipic acid,* which was found to possess the specific rotation $[\alpha]_D^{25} - 3.2°$ (chloroform, *c*, 5.9). In water (*c*, 10.75) the rotation was $[\alpha]_D^{22} - 2.2°$. The acid appears to form a molecular compound with benzene in the molecular ratio 2: 1. The solvent-free acid melts at 100.0–100.4°, and the same melting point is found for material obtained by crystallization from water.

The synthesis of the two dextrorotatory diastereoismers of 3,4-dimethyldocosanoic acid was carried out as shown in Charts I and II respectively. In order to show the steric connections between the compounds now synthesized the formulae in the charts have been written in the manner employed in the Fischer convention, but it must be emphasized that the choice of formula I a and II a respectively to represent the two active half-esters used as initial material is purely arbitrary.

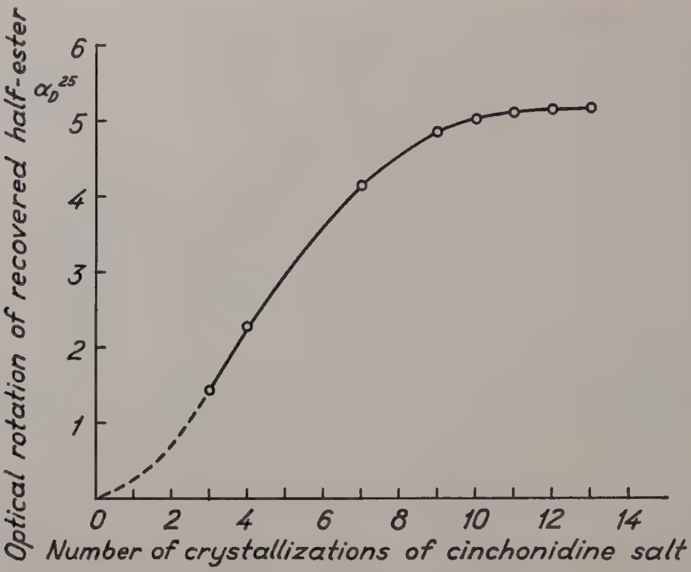


Fig. 1.

The lengthening of the chain of the two active half-esters was carried out by mixed electrolysis of their sodium salts and sodium stearate in ethanol-methanol solution. The general procedure was similar to that described in Parts VI (4) and VII (2). However, it was found more convenient to use a mixture of one part of methanol and two parts of ethanol than either of these alcohols alone as solvent. With pure methanol solubility difficulties are encountered, in that both the long chain hydrocarbon (*n*-tetratriacontane) formed and the sodium stearate are poorly soluble. On the other hand, if pure ethanol is used, the resistance of the electrolysis cell becomes large, and the current density has to be reduced in order to prevent too vigorous boiling of the electrolysis mixture.

Laevorotatory half-ester of *meso*-3,4-dimethyladipic acid gave in this manner a *dextrorotatory methyl 3,4-dimethyldocosanoate* (VI), b. p. 162°, 0.1 mm, $[\alpha]_D^{24} + 5.62°$ (homogeneous, supercooled liquid), $[M]_D^{24} + 21.5°$, in yields of 23–28 % of the theoretical. The solidified ester melts at 29.0–29.4°. Hydrolysis of the methyl ester gave a *dextrorotatory 3,4-dimethyldocosanoic acid*, $[\alpha]_D^{24} + 8.53°$, $[M]_D^{24} + 31.3°$. The acid melts at 50.4–50.6°, and the corresponding amide at 95.1–95.4°.

Lengthening the chain of the (dextrorotatory) mono-methyl ester of (—)-3,4-dimethyladipic acid in an analogous manner gave the second *dextrorotatory* diastereoisomer of *methyl 3,4-dimethyldocosanoate* (IX), b. p. 162–164°, 0.1 mm, $[\alpha]_D^{24} + 6.52°$ (homogeneous), $[M]_D^{24} + 24.9°$, in yields of 27–30 % of the theoretical. This methyl ester is dimorphous with a stable high melting form of m. p. 35.9–36.3° and an unstable low melting form of m. p. 20.1–20.2°. Hydrolysis of the methyl ester gave the *second dextrorotatory stereoisomer of 3,4-dimethyldocosanoic acid* (X), $[\alpha]_D^{24} + 5.34°$ (chloroform, *c*, 8.61), $[M]_D^{24} + 19.7°$. The acid possesses two crystalline modifications with melting points of 49.6–49.9° and

*Table I*

Molecular rotations at 25°.

| Compound | Wavelength, Å | | | | | |
|---|---|---|---|---|---|---|
| | 3131 | 3650 | 4046 | 4358 | 5461 | 5893 |
| (−)-Methyl hydrogen 3,4-dimethyladipate derived from the *meso*-3,4-dimethyladipic acid (homogeneous) | | −9.67 | −7.00 | −5.63 | −3.11 | −2.56 |
| (+)-Methyl hydrogen 3,4-dimethyladipate derived from (−)-3,4-dimethyladipic acid (homogeneous) | | +15.42 | +15.56 | +14.62 | +10.52 | +9.22 |
| (+)-3,4-Dimethyldocosanoic acid derived from *meso*-3,4-dimethyladipic acid (chloroform; *c*, 10.21) | +172 | +103.7 | +78.0 | +64.1 | +36.9 | +31.1 |
| (+)-3,4-Dimethyldocosanoic acid derived from (−)-3,4-dimethyladipic acid (chloroform; *c*, 9.12) | +68 | +53.7 | +43.8 | +37.5 | +23.1 | +19.6 |
| (−)-3,4-Dimethyladipic acid (chloroform; *c*, 5.91) | −73 | −33.3 | −21.4 | −16.1 | −7.5 | −5.5 |
| (−)-3,4-Dimethyladipic acid (water; *c*, 5.94) | | −25.2 | −15.7 | −11.3 | −4.7 | −4.0 |
| (+)-3,4,7,8-Tetramethylsebacic acid derived from *meso*-3,4-dimethyladipic acid (chloroform; *c*, 6.02) | +305 | +189 | +141.5 | +116.6 | +67.5 | +57.3 |
| (+)-3,4,7,8-Tetramethylsebacic acid derived from (−)-3,4-dimethyladipic acid (chloroform; *c*, 6.37) | +112 | +89.4 | +72.7 | +62.2 | +38.7 | +32.9 |

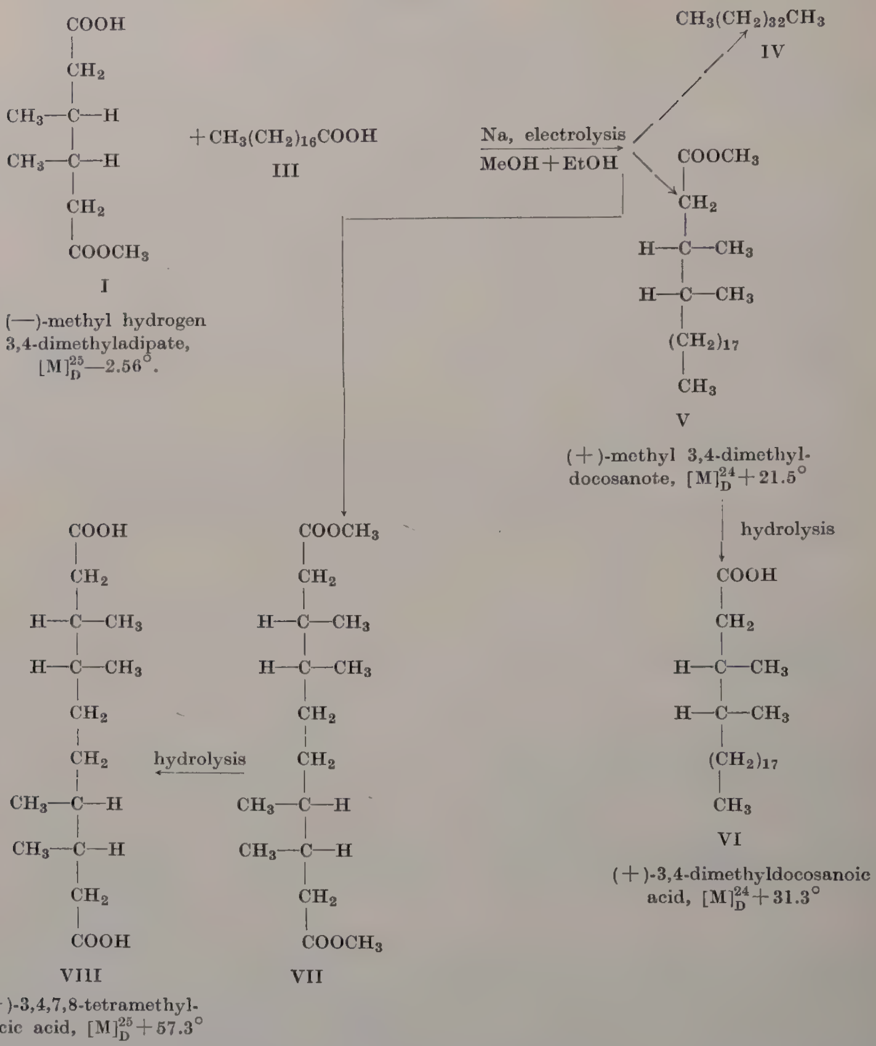
45.4–45.7° respectively. The *amide* is also dimorphous with melting points at 82.4–82.8° and 83.4–83.7° respectively.

In the electrolysis involving the laevorotatory half-ester derived from *meso*-3,4-dimethyladipic acid the *methyl ester* (VII) of the optically active 3,4,7,8-tetramethylsebacic acid (VIII) is obtained as by-product. After purification by column distillation the ester (VII) (b. p. 117.5°, 0.2 mm) had an optical rotation $[\alpha]_D^{24} + 18.0°$ (hom.) The *acid* (VIII), obtained by hydrolysis of the ester, melted at 74.5–75.5° and had $[\alpha]_D^{25} + 22.2°$ (chloroform; *c*, 6.02), $[M]_D^{25} + 57.3°$.

The corresponding *methyl ester* (XI) obtained from the electrolysis of the dextrorotatory monomethyl ester (II) of (−)-3,4-dimethyladipic acid distilled at 126°, 0.3 mm, and had $[\alpha]_D^{25} + 15.76°$ (hom.). The corresponding *dextrorotatory isomeride* (XII) of 3,4,7,8-tetramethylsebacic acid melted at 77.8–78.6° and had $[\alpha]_D^{25} + 12.7°$ (chloroform; *c*, 6.37), $[M]_D^{25} + 32.9°$.

Rotatory dispersion data for several of the optically active compounds prepared are given in Table I.

Chart I.



I

(—)-methyl hydrogen 3,4-dimethyladipate, $[M]_D^{25}$—2.56°.

III

IV

V

(+)-methyl 3,4-dimethyldocosanote, $[M]_D^{24}+21.5°$

VI

(+)-3,4-dimethyldocosanoic acid, $[M]_D^{24}+31.3°$

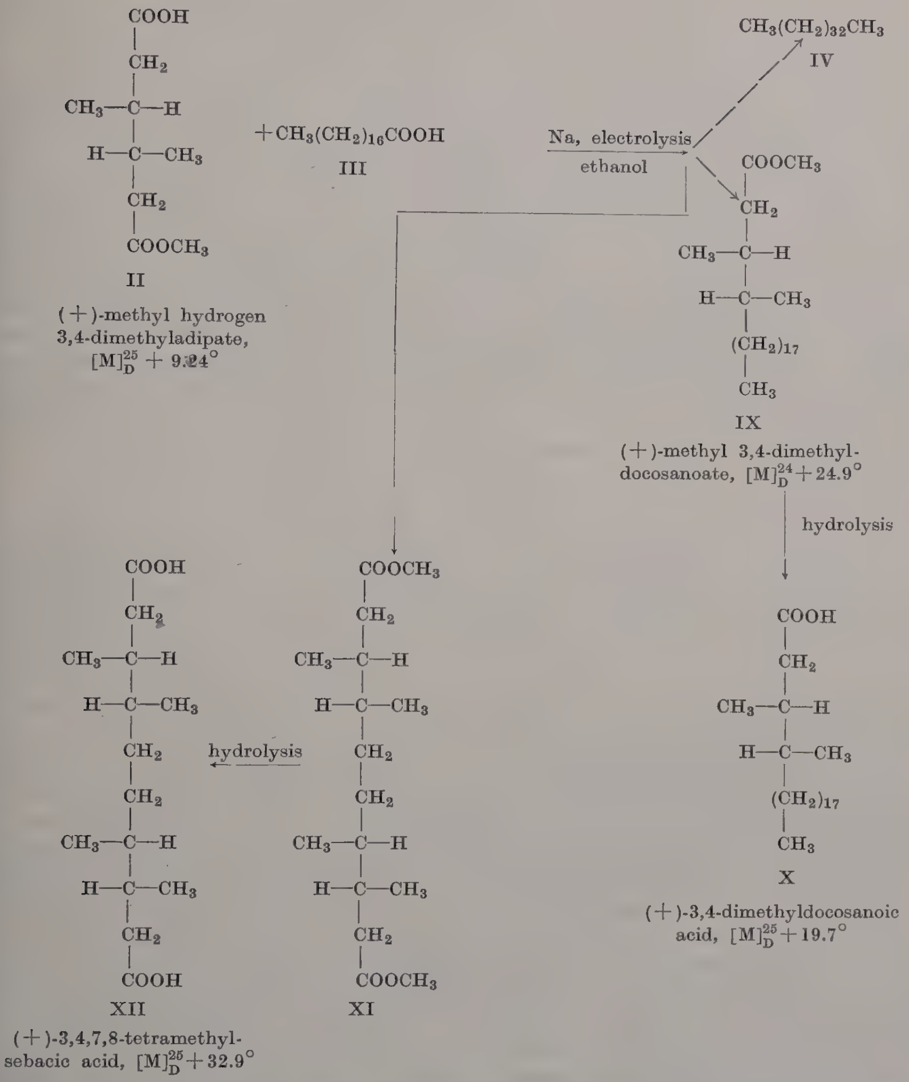
VII

VIII

(+)-3,4,7,8-tetramethylsebacic acid, $[M]_D^{25}+57.3°$

If the molecular rotations of D(+)-3-methyltricosanoic acid and D(+)-4-methyltetracosanoic acid described in Part VIII (7) are combined to give a calculated figure for the molecular rotations of 3,4-dimethyl-substituted long chain acids the figures obtained for the enantiomorphic pair derived from *meso*-3,4-dimethyladipic acid are ± 17°, and those for the corresponding pair derived from DL-3,4-dimethyladipic acid ± 14°. A comparison with the experimentally found values given above shows that in this case, when the active centres are in α-position to each other, the optical rotations cannot be calculated by simple addition. Furthermore, it is shown in Part X (8) that degradation of the dextrorotatory 3,4-dimethyldocosanoic acid derived from (—)-

Chart II.


COOH
CH2
CH3—C—H
H—C—CH3
CH2
COOCH3
II
(+)-methyl hydrogen 3,4-dimethyladipate, [M]25D + 9.24°
+CH3(CH2)16COOH
III
Na, electrolysis
ethanol
CH3(CH2)32CH3
IV
COOCH3
CH2
CH3—C—H
H—C—CH3
(CH2)17
CH3
IX
(+)-methyl 3,4-dimethyldocosanoate, [M]24D + 24.9°
hydrolysis
COOH
CH2
CH3—C—H
H—C—CH3
(CH2)17
CH3
X
(+)-3,4-dimethyldocosanoic acid, [M]25D + 19.7°
COOCH3
CH2
CH3—C—H
H—C—CH3
CH2
CH2
CH3—C—H
H—C—CH3
CH2
COOCH3
XI
hydrolysis
COOH
CH2
CH3—C—H
H—C—CH3
CH2
CH2
CH3—C—H
H—C—CH3
CH2
COOH
XII
(+)-3,4,7,8-tetramethylsebacic acid, [M]25D + 32.9°

3,4-dimethyladipic acid gives a 2,3-dimethylheneicosanoic acid with a rotation with the same sign, while according to the simple addition rule these two acids would be expected to show rotations of different signs.

## Experimental[1]

*Preparation of the mixture of stereoisomeric 3,4-dimethyladipic acids.* a) *By the method of* Faltis and Wagner. β-Bromobutyric acid was prepared as described by Lovén and Johansson (10), and the bromo-acid (b. p. 107–108°, 10 mm) converted into the iodo-acid by means of sodium iodide in acetone solution. The β-iodobutyric acid was purified by distillation under reduced pressure.

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[1] Melting points, crystal spacings and optical rotations were measured as described in Parts I (9) and VII (2).

The reaction between the iodo-acid and copper bronze was carried out in a rocking steel reaction vessel of one litre capacity, of the type used for high pressure hydrogenations (cf. (11)). 350 G of copper bronze, 250 g of iodo-acid, 150 g of crotonic acid, and 250 g of finely powdered pumice were used in each run. The temperature was kept at 110° for the first four hours, then at 135° for two hours, and finally at 145° during two hours. After cooling, the bomb contents were extracted three times with boiling water. The water solution was extracted with ether in an apparatus for continuous extraction. The ether solution was dried (sodium sulphate), the solvent distilled off, and the residue esterified by methanol and concentrated sulphuric acid in the usual manner. Distillation of the esterified material gave after a fore-run, mainly consisting of methyl crotonate, a mixture of the esters of the stereoisomeric 3,4-dimethyladipic acids boiling at 114–115°, 8 mm. The average yield was 53 % of the theoretical. The mixture of acids obtained on hydrolysis of the mixed esters usually melted in the region 90–95° before recrystallization.

b) *By the method of* Vogel. Ethyl ethylidenemalonate was prepared by the method of Goss, Ingold and Thorpe (12). 250 G of ethyl malonate, 250 g of acetic anhydride, and 140 g of acetaldehyde were used in each run. The mixture was kept at 100° for 24 hours in the steel reaction vessel mentioned above (no rocking was used in this case). Distillation through a 15 cm electrically heated, glass packed column gave ethyl ethylidenemalonate, b. p. 97–98°, 8 mm, in about 85 % yield. It was found best to distil off the fore-run under reduced pressure.

250 G of aluminium foil about 0.05 mm thick were cut in strips about 2 cm wide and amalgamated as described by Vogel (13). 5.5 Litres of moist ether were added to the amalgamated aluminium foil in a round-bottomed flask of 10 litres capacity, fitted with two effective reflux condensers and one dropping funnel. The ethyl ethylidenemalonate (275 g) was added through the dropping funnel at such a rate that the reaction mixture did not boil too vigorously. If necessary, the flask was cooled with cold water. When the reaction showed signs of slowing down, 200 ml of water were added and the mixture left standing for three hours with occasional stirring. After this time most of the aluminium had reacted. Dilute sulphuric acid (800 ml of concentrated acid in 2000 ml of water) was slowly added through the dropping funnel with stirring and external cooling. The mixture was transferred to a large separating funnel and the ether layer separated. The water layer was extracted with ether. The combined ether solutions were washed with water and dried with sodium sulphate. The solvent was distilled off and the residue distilled *in vacuo*. After fore-runs distilling up to 152°, 0.5 mm, ethyl 2,3-dimethylbutane-1,1,4,4-tetracarboxylate (205 g or 75 % of the theoretical), distilled at 152–155°, 0.5 mm. The yields varied in different runs between 63 and 75 % of the theoretical. A mixture of the tetra-ester (450 g), potassium hydroxide (450 g), water (900 ml) and methanol (900 ml) was refluxed for 8 hours. About 1000 ml was distilled off from the hydrolysis mixture, 500 ml of water added and the distillation continued (finally under slightly reduced pressure) until practically all methanol and ethanol had

been removed. The residue was acidified with an excess of dilute hydrochloric acid, and the solution extracted with ether in an apparatus for continuous extraction. The ether solution was dried with sodium sulphate, the solvent distilled off, and the residue subjected to decarboxylation by heating gradually to 200°. Decarboxylation evidently takes place to some extent already during the hydrolysis, as an oil layer forms on top of the solution and a strong evolution of carbon dioxide occurs on acidification of the hydrolysis mixture. The oil has a very characteristic smell, and apparently consists of cyclic ketone formed by further decarboxylation and ring closure.

The mixture obtained after decarboxylation was esterified with methanol and sulphuric acid. The yield of methyl ester of the stereoisomeric 3,4-dimethyladipic acids was 124 g or 51 % of the theoretical, calculated on the tetra-ester. The yield could not be improved by changing the hydrolysis conditions for the tetra-ester. Hydrolysis of the mixture of dimethyl esters gave a mixture of 3,4-dimethyladipic acids melting at 117–125°, the yield being 90–95 % of the theoretical.

meso-*2,3-Dimethylbutane-1,1,4,4-tetracarboxylic acid.* Recrystallization from acetone of a mixture of the tetracarboxylic acids obtained by the method of VOGEL gave the pure higher melting stereoisomer of m. p. 185°. (VOGEL (6) gives m. p. 185–185.5°). Decarboxylation of this specimen gave pure high melting 3,4-dimethyladipic acid (m. p. 133° without recrystallization). As this acid is the *meso*-form (cf below) it follows that the higher melting tetracarboxylic acid is the *meso*-acid.

meso-*3,4-Dimethyladipic acid.* Two to three recrystallizations from water or from ether of the mixture of acids obtained by the method of VOGEL gave the pure higher melting acid of m. p. 133–134°. In order to obtain a specimen of the higher melting acid of the same purity from the mixture obtained by the method of FALTIS and WAGNER, six to seven recrystallizations were necessary. Hydrolysis of the laevorotatory methyl half-ester of the high melting acid gave a completely inactive acid of m. p. 133–134°, indicating that the higher melting of the two stereoisomers is the *meso*-compound.

DL-*methyl hydrogen 3,4-dimethyladipate derived from* meso-*acid* (I). The half-ester of the *meso*-acid was prepared by partial esterification of the acid (240 g of m. p. 133–134°) with methanol (80 ml) and concentrated hydrochloric acid (25 ml). The mixture was refluxed for 5 hours. After cooling, ether was added and the water layer separated. The water layer was extracted once with ether, and the combined ether solutions were dried with sodium sulphate. The solvent was distilled off, and the residue distilled through a 15 cm electrically heated and packed column. The following fractions were collected.

| | | | |
|---|---|---|---|
| I | 72°, | 0.5 mm | 76 g |
| II | 72–104°, | 0.3 » | 6 » |
| III | 104–106°, | 0.3 » | 116 » |

A solid residue weighing about 40 g was left in the flask. Fraction III consists of the desired half-ester.

*Anal.*: Calcd for $C_9H_{16}O_4$ (188.2) C, 57.43; H, 8.57 %
Found C, 57.34; H, 8.64 %

Fraction I consists of methyl *meso*-3,4-dimethyladipate.

*Anal.*: Calcd for $C_{10}H_{18}O_4$ (202.2) C, 59.38; H, 8.98 %
Found C, 59.01; H, 9.02 %

Partial saponification of the dimethyl ester gave the half-ester in yields of 50–55 % of the theoretical.

*Laevorotatory enantiomorph of the monomethyl ester* (I) *of* meso-*3,4-dimethyladipic acid.*

It was found in preliminary experiments that the cinchonidine salt of the half-ester readily crystallized from 60 % acetone at 0°. No quinine salt was formed under similar conditions, and the experiments with quinine were not further pursued. DL-half-ester (85 g) was dissolved in 60 % acetone (800 ml) and cinchonidine (132 g) added. The mixture was warmed and stirred until the cinchonidine had dissolved. The solution was filtered while still hot and left overnight in the refrigerator at 0°. The cinchonidine salt crystallized in the form of large rosettes of long, thin needles. Part of the salt was decomposed with dilute hydrochloric acid, and the recovered half-ester showed after distillation on optical rotation $\alpha_D^{24} -0.98°$ (homogeneous; *l*, 1). The salt was recrystallized from 300 ml of 60 % acetone in the cold, and a specimen of recovered half-ester now had $\alpha_D^{23} -1.35°$. After a further crystallization from 200 ml of 60 % acetone the recovered half-ester had $\alpha_D^{23} -1.49°$ (homogeneous; *l*, 1). Further recrystallization did not change the rotation of the half-ester. The yield of cinchonidine salt after three crystallizations was 30 g. The salt was decomposed by adding an excess of dilute hydrochloric acid. The half-ester was extracted with ether, and the ether solution washed carefully with water to remove all hydrochloric acid. The ether solution was dried with sodium sulphate and the solvent distilled off. The residue was distilled under reduced pressure, giving 9.9 g (23 % of the theoretical) of *laevorotatory monomethyl ester* of meso-*3,4-dimethyladipic acid.* The conditions for optimum yield are fairly critical, and the yields obtained in different runs differed considerably. Sometimes more than three crystallizations were needed to bring the rotation to a constant value, and in such cases the yields were only about 10 % of the theoretical. $d_4^{20}$ 1.079. $d_4^{25}$ 1.075.

*Optical rotation* $\alpha_D^{20} -1.51°$ (homogeneous; *l*, 1)
$[\alpha]_D^{20} -1.40°$ $[M]_D^{20} -2.64°$
$\alpha_D^{25} -1.465°$ (homogeneous; *l*, 1)
$[\alpha]_D^{25} -1.36°$ $[M]_D^{25} -2.56°$

*Anal.*: Calcd for $C_9H_{16}O_4$ (188.2) C, 57.43; H, 8.57 %
Found C, 57.40; H, 8.50 %

*Dextrorotatory monomethyl ester* (II) *of* (—)-*3,4-dimethyladipic acid.* The mixed methyl esters of the 3,4-dimethyladipic acids obtained by the method of FALTIS and WAGNER were hydrolyzed. The free acids were dissolved in hot water (500 ml of water for 100 g of acid), the solution allowed to cool slightly, seeded with powdered pure *meso*-acid, and left at room temperature. The solution was stirred occasionally during the crystallization. After standing for 24 hours the crystals were separated from the mother liquor. The crystals usually represented half of the initial material. The mother liquors from several runs were combined and extracted several times with ether. The ether solution was dried with sodium sulphate, and the solvent distilled off. The residue, which consisted of DL-3,4-dimethyladipic acid together with some *meso*-acid, was converted into monomethyl ester by partial esterification (the half-ester was also prepared by partial hydrolysis of dimethyl ester from previous runs).

The monomethyl ester (142 g) was dissolved in 2200 ml of 60 % acetone, 222 g of cinchonidine added, and the mixture warmed and stirred until the alkaloid had dissolved. The solution was filtered hot and left in the refrigerator at 0° overnight. The cinchonidine salt crystallized in the form of fairly large, thick crystals. After twelve crystallizations from successively smaller volumes of 60 % acetone, the rotation of the recovered half-ester had reached a constant value. Crystals of the cinchonidine salt from the later crystallizations were in the form of thin prisms, and differed considerably in appearance from those obtained in the initial stages of the resolution.

The course of the resolution is shown in Fig. 1.

The yield of *(+)-methyl hydrogen 3,4-dimethyladipate derived from laevorotatory 3,4-dimethyladipic acid* was 11.6 g. The mother liquors were employed in the crystallizations of the later runs. From a total amount of 354 g of DL-half-ester (containing some half-ester from the *meso*-acid), 31.4 g (about 20 % of the theoretical) of pure dextrorotatory half-ester were obtained. $d_4^{20}$ 1.078. $d_4^{25}$ 1.075.

*Optical rotation* $\alpha_D^{20} + 5.35°$ (homogeneous; *l*, 1)
$[\alpha]_D^{20} + 4.97°$ $[M]_D^{20} + 9.35°$
$\alpha_D^{25} + 5.28°$ (homogeneous; *l*, 1)
$[\alpha]_D^{25} + 4.91°$ $[M]_D^{25} + 9.24°$

*Anal.*: Calcd for $C_9H_{16}O_4$ (188.2) C, 57.43; H, 8.57 %
Found C, 57.30; H, 8.50 %

DL-3,4-*Dimethyladipic acid.* All attempts to prepare the lower melting acid by direct crystallization from different solvents in the manner described by FALTIS and WAGNER (5) failed to give a pure product. A pure specimen of the racemic methyl ester was therefore prepared by mixing 3.57 g of partially resolved (+)-methyl 3,4-dimethyladipate (related to the DL-acid), $\alpha_D^{23} + 1.82°$ (homogeneous; *l*, 1) with 3.87 g of partially resolved laevorotatory half-ester, having $\alpha_D^{24} - 1.68°$ (homogeneous; *l*, 1). The laevorotatory half-ester was obtained from the mother liquor from the 7th recrystallization of one of the later runs in the preparation of the dextrorotatory half-ester. At this stage of the fractional crystallization process, half-ester from *meso*-acid had been removed, and the repeated use of mother liqours from the earlier runs had led to an accumulation of the laevorotatory half-ester. The racemic ester thus obtained was hydrolyzed, and the free acid crystallized from water. The DL-3,4-dimethyladipic acid was obtained in the form of rather thick, transparent crystals, melting at 115.5–116.5°. The same m. p. was found for a previously melted specimen that had been cooled to room temperature. On reheating immediately after solidification of the melt, melting occurred at 107–108°, indicating the existence of an unstable low-melting modification.

(—)-*3,4-Dimethyladipic acid.* Hydrolysis of the dextrorotatory monomethyl ester just described gave *laevorotatory 3,4-dimethyladipic acid.* The acid crystallized from benzene in the form of beautiful, transparent, lustrous needles, which after a few hours in air turned opaque. Fresh crystals melted at about 85°, but the melting point was very diffuse. It was found that the crystals, which appeared quite dry, on keeping slowly gave off benzene. 0.298 G of fresh crystals after one hour in a vacuum desiccator weighed 0.289 g. After further keeping in air for 5 hours the weight was 0.265 g, and finally after 48 hours in air 0.248 g. The loss corresponds approximatively to one mole of benzene for two moles of acid. (1:2.18). The benzene-free, opaque crystals melted at 100.0–100.4°. Solidification took place at 99.8° with the formation of beautiful long prisms within the melt, and the acid remelted at 100.0–100.4°. The acid is fairly soluble in water at room temperature. Crystallization from water gave (—)-3,4-dimethyladipic acid in the form of transparent thick prisms with the same m. p. as the benzene-free material just mentioned. The two specimens also gave identical X-ray diffraction patterns. Fresh crystals from benzene gave an entirely different diffraction pattern. If benzene-free crystals (originally obtained from benzene) were moistened with benzene some swelling took place, and the X-ray diffraction pattern changed to that of the benzene-containing crystals. It thus appears that (—)-3,4-dimethyladipic acid forms a molecular compound with benzene.

*Optical rotation* $\alpha_D^{25}$ —0.38° (chloroform; *l*, 2; *c*, 5.91)
$[\alpha]_D^{25}$ —3.2° $[M]_D^{25}$ —5.5°
$\alpha_D^{25}$ —0.27° (water; *l*, 2; *c*, 5.94)
$[\alpha]_D^{25}$ —2.3° $[M]_D^{25}$ —4.0°

*Anal.*: Calcd for $C_8H_{14}O_4$ (174.2) C, 55.16; H, 8.10 %
Found C, 55.28; H, 8.17 %

(+)-*Methyl 3,4-dimethyladipate* was obtained by esterification of the dextrorotatory half-ester by methanol and sulphuric acid. The ester, a fairly mobile, colourless liquid, distilled at 76°, 0.6 mm. $d_4^{22}$ 1.029.

*Optical rotation* $\alpha_D^{22}$ + 8.04° (homogeneous; *l*, 1)
$[\alpha]_D^{22}$ + 7.81° $[M]_D^{22}$ + 15.80°

*Anal.*: Calcd for $C_{10}H_{18}O_4$ (202.2) C, 59.38; H, 8.98 %
Found C, 59.53; H, 9.00 %

(+)-*Methyl 3,4-dimethyldocosanoate* (V) derived from *meso*-3,4-dimethyladipic acid.

Laevorotatory monomethyl ester of *meso*-3,4-dimethyladipic acid (I) (6.1 g, 0.0324 mole) and stearic acid (III) (9.2 g, 0.0324 mole, the specimen used was the one described in Part VII (2)) were added to a solution of sodium (0.4 g) in methanol-ethanol (300 ml). The electrolysis was carried out between platinum electrodes in the manner described in Part VII (2). When the solution was neutral, 6.1 g of active half-ester and 9.2 g of stearic acid were added, and the electrolysis continued until the solution again became neutral. The mixture was allowed to cool to room temperature. The crystalline *n*-tetratriacontane was filtered off and washed on the filter with methanol. Water was added to the filtrate, and the mixture extracted with ether. The ether solution was washed with 5 % sodium carbonate solution, and the precipitated sodium stearate removed. The treatment with sodium carbonate was repeated until no more precipitate was obtained. The ether solution was washed with water, dried with sodium sulphate, and the solvent distilled off under reduced pressure. The residue was distilled in a small all-glass CLAISEN type apparatus. The following fractions were collected.

| | | | | |
|---|---|---|---|---|
| I | up to 91°, | 0.1 mm | 1 g | |
| II | 91–108°, | 0.1 » | 3.56 » | $\alpha_D^{23} + 12.08°$ (hom.; *l*, 1) |
| III | 108–162°, | 0.1 » | 1 » | |
| IV | 162°, | 0.1 » | 5.58 » | $\alpha_D^{23} + 4.86°$ (hom.; *l*, 1) |

Fraction IV consists of the desired *methyl ester* of *3,4-dimethyldocosanoic acid* (V), while fraction II consists mainly of the *methyl ester* (VII) of one of the diastereoisomers of 3,4,7,8-tetramethylsebacic acid (VIII). The methyl ester (V) solidified on standing to a beautifully crystalline solid, which melted at 28.6–29.1°. Crystallization from methanol gave lustrous plates of m. p. 29.0–29.4°. $d_4^{24}$ 0.864 (supercooled liquid).

Long X-ray spacing (from methanol) 29.9 Å.

*Optical rotation* $\alpha_D^{24} + 4.86°$ (homogeneous (supercooled liquid)); *l*, 1)
$[\alpha]_D^{24} + 5.62°$ $[M]_D^{24} + 21.5°$

*Anal.*: Calcd for $C_{25}H_{50}O_2$ (382.7) C, 78.47; H. 13.17 %
Found C, 78.33; H, 13.01 %

(+)-*3,4-Dimethyldocosanoic acid* (VI) derived from *meso*-3,4-dimethyladipic acid. The methyl ester just described (4.3 g) was hydrolyzed by refluxing for 4 hours with potassium hydroxide (10 g) in water (7 ml) and ethanol (125 ml). After acidification the free acid was taken up in ether in the usual manner. Crystallization from methanol gave 3.74 g (90 % of the theoretical) of (+)-*3,4-dimethyldocosanoic acid* (VI) derived from *meso*-3,4-dimethyladipic acid. The acid formed small lustrous plates which melted at 50.4–50.6°. The solidified acid remelted at 50.4–50.6°. Long X-ray spacing (pressed specimen) 31.8 Å. Some very faint lines corresponding to a spacing of 25.8 Å were also present on the photograph.

*Optical rotation* $\alpha_D^{24} + 2.90°$ (chloroform; *l*, 4; *c*, 8.55)
$[\alpha]_D^{24} + 8.53°$ $[M]_D^{24} + 31.3°$

*Anal.*: Calcd for $C_{24}H_{48}O_2$ (368.6) C, 78.19; H, 13.13 %
Found C, 78.57; H, 13.11 %

The *amide* of the acid just described was prepared via the acid chloride. After crystallization from methanol or from acetone the amide melted at 82.4–82.8°. After solidification of the melt, remelting occurred at 83.4–83.7°, indicating that the amide is dimorphous. The amide crystallizes from methanol in the form of lustrous prismatic needles.

Long X-ray spacing (pressed specimen) 33.2 Å.

*Anal.*: Calcd for $C_{24}H_{49}ON$ (367.6) N, 3.82 %
Found N, 3.80 %

(+)-*Methyl 3,4,7,8-tetramethylsebacate* of the configuration (VII), derived from *meso*-3,4-dimethyladipic acid. Redistillation of the fore-runs (12.7 g) from the distillation of the electrolysis products through a 50 cm Podbielniak column gave the following fractions.

| | | | | |
|---|---|---|---|---|
| I | Up to 103°, | 0.2 mm | 0.4 ml | |
| II | 103–104°, | 0.2 » | 3 » | $\alpha_{D}^{22}$ + 3.40° (hom.; *l*, 1) |
| III | 104–117°, | 0.2 » | 2 » | $\alpha_{D}^{22}$ + 10.42° (hom.; *l*, 1) |
| IV | 117–117.5° | 0.2 » | 1.88 g | $\alpha_{D}^{24}$ + 17.34° (hom.; *l*, 1) |
| V | 117.5°, | 0.2 » | 1.82 » | $\alpha_{D}^{24}$ + 17.45° (hom.; *l*, 1) |
| VI | 117.5–121°, | 0.2 » | 0.51 » | $\alpha_{D}^{22}$ + 15.94° (hom.; *l*, 1) |

Fractions IV and V consist of the *methyl ester* (VII). $d_{4}^{24}$ 0.969.

*Optical rotation* $\alpha_{D}^{24}$ + 17.45° (homogeneous; *l*, 1)
$[\alpha]_{D}^{24}$ + 18.01° $[M]_{D}^{24}$ + 51.6°

*Anal.*: Calcd for $C_{16}H_{30}O_4$ (286.4) C, 67.10; H, 10.56 %
Found C, 67.47; H, 10.56 %

(+)-*3,4,7,8-Tetramethylsebacic acid* (VIII) derived from *meso*-3,4-dimethyladipic acid. The acid was obtained by alkaline hydrolysis of the ester described above. It crystallized from benzene-light petroleum (b. p. 40–60°) (1:3 by volume) as aggregates of transparent short prisms. The melting point of the crystallized acid was 75–76°. A previously melted specimen had the same m. p.

*Optical rotation* $\alpha_{D}^{25}$ + 2.65° (chloroform; *l*, 2; *c*, 6.02)
$[\alpha]_{D}^{25}$ + 22.0° $[M]_{D}^{25}$ + 56.8°

*Anal.*: Calcd for $C_{14}H_{26}O_4$ (258.4) C, 65.08; H, 10.14 %
Found C, 64.74; H, 10.13 %

(+)-*Methyl 3,4-dimethyldocosanoate* (IX) derived from (−)-3,4-dimethyladipic acid.

The electrolysis was carried out in the same manner as in the synthesis of the stereoisomer, with a total amount of 10.9 g of (+)-methyl hydrogen 3,4-dimethyladipate and 16.4 g of stearic acid. The electrolysis mixture was worked up in the manner already described above, and distillation of the product gave the following fractions.

| | | | | |
|---|---|---|---|---|
| I | up to 90°, | 0.1 mm | 0.6 g | |
| II | 90–103°, | 0.1 » | 4.2 » | $\alpha_{D}^{23}$ + 9.43° (hom.; *l*. 1) |
| III | 103–162°, | 0.1 » | 0.5 » | |
| IV | 162–164°, | 0.1 » | 6.05 » | $\alpha_{D}^{24}$ + 5.63° (hom.; *l*, 1) |

All fractions were colourless.

Fraction II consists mainly of the *methyl ester* of the tetramethylsebacic acid (VIII), and fraction IV is the *methyl ester* (V) of the desired second dextrorotatory diastereoisomer of 3,4-dimethyldocosanoic acid of the configuration given by formula (VI). The methyl ester (V) after solidification at room temperature formed a crystalline solid which melted at 35.9–36.3°. On rapid cooling of the melt, solidification to a beautifully crystalline solid took place at 19.9°. On reheating, melting occurred at 20.1–20.2°. The methyl ester thus exhibits dimorphism.

Long X-ray spacing (high melting form) 30.1 Å.

*Optical rotation* $\alpha_D^{24} + 5.63^\circ$ (homogeneous (supercooled liquid); *l*, 1)
$[\alpha]_D^{24} + 6.52^\circ$ $[M]_D^{24} + 24.9^\circ$

*Anal.*: Calcd for $C_{25}H_{50}O_2$ (382.7) C, 78.47; H, 13.17 %
Found C, 78.58; H, 13.16 %

(+)-*3,4-Dimethyldocosanoic acid* (X) derived from (—)-3,4-dimethyladipic acid. Hydrolysis of the methyl ester (V) gave the acid (VI) which after crystallization from acetone melted at 49.6–49.9°. After previous melting the m. p. was 45.4–45.7°. The higher melting modification was obtained from acetone in the form of small lustrous needles.

Long X-ray spacing (pressed specimen) 22.6 Å.

*Optical rotation* $\alpha_D^{25} + 1.84^\circ$ (chloroform; *l*, 4; *c*, 8.6)
$[\alpha]_D^{25} + 5.34^\circ$ $[M]_D^{25} + 19.7^\circ$

*Anal.*: Calcd for $C_{24}H_{48}O_2$ (368.6) C, 78.19; H, 13.13 %
Found C, 78.06; H, 13.13 %

The *amide* of the dextrorotatory 3,4-dimethyldocosanoic acid (IX) crystallized from methanol in the form of elongated, thin lustrous plates of m. p. 95.1–95.4°. After solidification of the melt the remelting occurred at 94.9–95.3°.

Long X-ray spacing (pressed specimen) 33.4 Å.

*Anal.*; Calcd for $C_{24}H_{49}ON$ (367.6) N, 3.82 %
Found N, 3.77 %

(+)-*Methyl 3,4,7,8-tetramethylsebacate* (XI) derived from (—)-3,4-dimethyladipic acid. The methyl ester (11 g) obtained from the electrolysis experiments was redistilled through a 50 cm Podbielniak type column. The following colourless fractions were collected.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| I | up to 100°, | 0.2 | mm | 0.4 | g | | |
| II | 100–102°, | 0.2 | » | 1.34 | » | $\alpha_D^{24} + 0.70^\circ$ | (hom.; *l*, 1) |
| III | 102–113°, | 0.2 | » | 1.84 | » | $\alpha_D^{24} + 1.66^\circ$ | (hom.; *l*, 1) |
| IV | 113–124°, | 0.3 | » | 1.18 | » | $\alpha_D^{24} + 9.56^\circ$ | (hom.; *l*, 1) |
| V | 124–126°, | 0.3 | » | 2.24 | » | $\alpha_D^{25} + 15.00^\circ$ | (hom.; *l*, 1) |
| VI | 126°, | 0.3 | » | 1.70 | » | $\alpha_D^{25} + 15.26^\circ$ | (hom.; *l*, 1) |
| VII | 126°, | 0.3 | » | 1.27 | » | $\alpha_D^{25} + 15.26^\circ$ | (hom.; *l*, 1) |

Fractions II and III consist mainly of heptadecene-1 and *n*-heptadecane formed as a by-product from the stearic acid during the electrolysis. The saturated hydrocarbon is formed by catalytic hydrogenation of the unsaturated hydrocarbon at the cathode surface. Fractions VI and VII consist of the pure methyl ester (XI). The latter has $d_4^{25}$ 0.968.

*Optical rotation* $\alpha_D^{25} + 15.26^\circ$ (homogeneous; *l*, 1)
$[\alpha]_D^{25} + 15.76^\circ$ $[M]_D^{25} + 45.1^\circ$

*Anal.*: Calcd for $C_{16}H_{30}O_4$ (286.4) C, 67.10; H, 10.56 %
Found C, 67.08; H, 10.54 %

(+)-*3,4,7,8-Tetramethylsebacic acid* with the configuration (XII) (derived from (—)-3,4-dimethyladipic acid) was obtained by hydrolysis of the methyl ester (VII). Crystallization from a mixture of benzene and light petroleum (b. p. 40–60°) (1:3 by volume) gave the tetramethylsebacic acid (XII) in the form of aggregates of stout transparent crystals. The acid melted at 77.8–78.6°, unchanged after previous melting.

*Optical rotation* $\alpha_D^{25} + 1.62^\circ$ (chloroform; *l*, 2; *c*. 6.37)
$[\alpha]_D^{25} + 12.7^\circ$ $[M]_D^{25} + 32.9^\circ$

*Anal.*: Calcd for $C_{14}H_{26}O_4$ (258.4) C, 65.08; H, 10.14 %
Found C, 65.30; H, 10.10 %

The author is indebted to Mr. L. AHLQUIST for assistance in the synthetic work. The analyses have been performed by Miss A. RENMAN and Mr. I. ALPEROWICZ in the microananalytical laboratory of this Institute. A large part of the expenses involved in this work have been defrayed by grants from the Swedish National Anti-tuberculosis Association.

## SUMMARY

A method of synthesizing long chain compounds with two optically active carbon atoms with methyl side chains adjacent to each other is described. The two stereoisomeric 3,4-dimethyladipic acids are used as initial material. The higher melting acid is shown to be the *meso*-form, and the laevorotatory enantiomorph of the methyl half-ester of this acid has been obtained via the cinchonidine salt. Lengthening of the chain of the active mono-ester was carried out by mixed electrolysis of its sodium salt with sodium stearate in alcoholic solution, giving a dextrorotatory methyl 3,4-dimethyldocosanoate, which on hydrolysis gave one of the dextrorotatory diastereoisomers of 3,4-dimethyldocosanoic acid.

The second dextrorotatory diastereoisomer of 3,4-dimethyldocosanoic acid was obtained in an analogous manner starting from the dextrorotatory methyl half-ester of (—)-3,4-dimethyladipic acid. The cinchonidine salts of the methyl half-esters of DL-3,4-dimethyladipic acid are considerably less soluble than those of the *meso*-acid, and use was made of this fact to obtain the methyl half-esters of the DL-acid free from *meso*-acid half-esters. Repeated crystallizations of the cinchonidine salts finally gave the dextrorotatory of the two enantiomorphic half-esters of the DL-acid. Hydrolysis and esterification of the active half-ester gave (—)-3,4-dimethyladipic acid and (+)-methyl 3,4-dimethyladipate respectively.

Two of the dextrorotatory diastereoisomers of 3,4,7,8-tetramethylsebacic acid have been prepared by hydrolysis of the methyl esters obtained as by-products in the electrolyses.

Rotatory dispersion data for several of the compounds prepared are given. The optical rotation of branched chain compounds with two active centres in α-position to each other cannot be obtained by adding the contributions of the separate centres using data from mono-substituted compounds with the active centres in the corresponding positions.

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REFERENCES: 1. **Ställberg-Stenhagen, S.,** Arkiv för Kemi *1,* 187 (1949). — 2. *Idem, ibid., 2,* 431 (1950). — 3. *Idem,* Arkiv för Kemi, Min. o. Geol. *26 A,* No. 1 (1948). — 4. *Idem,* Arkiv för Kemi *2,* 95 (1950). — 5. **Faltis, F.,** and **Wagner, H.,** Annalen *433,* 103 (1923). — 6. **Vogel, I.,** J. Chem. Soc. *1927,* 1985. — 7. **Ställberg-Stenhagen, S.,** Arkiv för Kemi, *3,* 117 (1951). — 8. *Idem, ibid., 3,* 267 (1951). — 9. *Idem,* Arkiv för Kemi, Min. o. Geol. *23 A,* No. 15 (1946). — 10. **Lovén, J. M.,** and **Johansson, H.,** Berichte *48,* 1256 (1915). — 11. **Adkins, H.,** Reactions of hydrogen, Madison 1937. — 12. **Goss, F. R., Ingold, C. K.,** and **Thorpe, J. F.,** J. Chem. Soc. *73,* 3342 (1923). — 13. **Vogel, I.,** J. Chem. Soc. *1927,* 594.

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# Optically active higher aliphatic compounds. X

## Degradation of the dextrorotatory 3,4-dimethyldocosanoic acid derived from (—)-3,4-dimethyladipic acid to a dextrorotatory 2,3-dimethylheneicosanoic acid

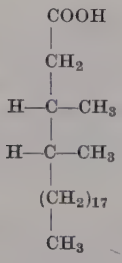
By STINA STÄLLBERG-STENHAGEN

The degradation of an optically active β-methyl substituted long chain acid to the sterically related α-methyl substituted acid with one carbon atom less was described in Part IV (1). It was of interest to attempt the same type of degradation on the optically active diastereoisomeric 3,4-dimethyldocosanoic acids described in Part IX (2), as this would appear to be the most convenient route to the difficultly accessible optically active 2,3-dimethyl substituted long chain acids.

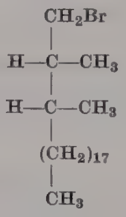
Degradation of the silver salt of (+)-3,4-dimethyldocosanoic acid of $[M]_D^{25} + 19.7$ (I) (derived from (—)-3,4-dimethyladipic acid) by bromine in carbon tetrachloride solution according to HUNSDIECKER and HUNSDIECKER (3,1) gave (+)-*1-bromo-2,3-dimethylheneicosane* (III) of m. p. 33.5—34.0°, $[\alpha]_D^{24} + 6.0°$. The yield of bromide was about 50 % of the theoretical. Prolonged refluxing of the bromide with potassium acetate in a mixture of glacial acetic acid and butyl acetate gave the acetate (IV) of the corresponding alcohol (V). The crude alcohol obtained by hydrolysis of the acetate was oxidized by potassium permanganate in acetone solution at room temperature. The crude acid (VI) thus obtained was purified via the barium salt. After further purification by recrystallization of the free acid from light petroleum and from acetone, a beautifully crystalline *dextrorotatory 2,3--dimethylheneicosanoic acid* of m. p. 65.8—66.1°, $[\alpha]_D^{23} + 18.6°$, $[M]_D^{23} + 66°$ was obtained. The yield was 18 % of the theoretical, calculated on the bromide (III). Both the melting point and the optical rotation of the 2,3-dimethyl substituted acid are higher than those of the parent 3,4-dimethyl substituted acid (I). The latter acid has m. p. 49.6—49.9° and 45.4—45.7° (two different crystalline forms) and $[M]_D^{25} + 19.7°$. The *amide* of the (+)-2,3-dimethylheneicosanoic acid also has a rather high m. p., *viz.* 118.1—118.3°.

The degradation of the second dextrorotatory diastereoisomer of 3,4-dimethyldocosanoic acid (VII) (derived from *meso*-3,4-dimethyladipic acid) in an analogous manner gave a *dextrorotatory bromide* (VIII) of m. p. 33.3—34.0°, $[\alpha]_D^{23} + 6.8°$. Conversion of this bromide into the corresponding 2,3-dimethylsubstituted acid by the series of reactions mentioned above did not give the expected product, however. In spite of many attempts at purification, the compound obtained had a melting range extending over several degrees, and the analytical data did not fit the $C_{23}H_{46}O_2$ formula, but agreed within the experimental error with the formula $C_{23}H_{46}O_3$. The optical rotation was low, $[\alpha]_D^{24} + 1.6°$. The most likely explanation of these findings seems to be that in this case the oxidation of the alcohol also leads to the introduction of a hydroxyl group in α-position to the carboxyl group, accompanied by partial or complete racemization of the active centre in this position. The hydroxy-acid produced is thus a mixture of diastereoisomers, which explains the melting behaviour.

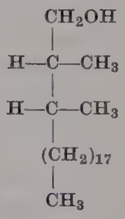
As the above results were obtained when the oxidation was carried out at 37°, it appeared possible that the reaction temperature was too high, but repetition of the experiment with the oxidation step performed at 20° gave an identical result. It is possible that hydroxy-acid is also formed as a by-product in the oxidation of the alcohol (V) derived from (—)-3,4-dimethyladipic acid, and that for steric reasons this by-product becomes the main product of the oxidation of the stereoisomer (IX). It is of interest in this connexion that α-hydroxy-*iso*butyric acid has been obtained in low yield by oxidation of *iso*butyric acid with alkaline permanganate (4).



VII

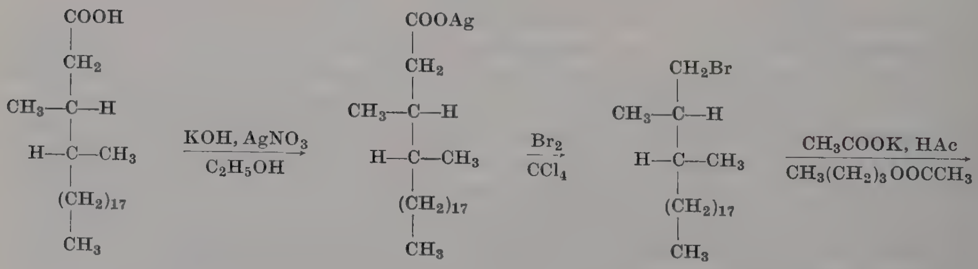


VIII



IX

Chart.[1]



I

II

III

(+)-3,4-dimethyldocosanoic acid, $[M]_D^{25} + 19.7°$

(+)-1-bromo-2,3-dimethylheneicosane, $[M]_D^{24} + 24°$

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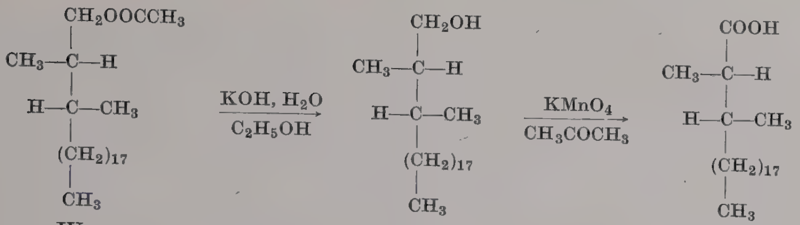
[1] The formulae are written in the manner employed in the FISCHER convention, but the assignment of formula (I) to (+)-3,4-dimethyldocosanoic acid derived from (—)-3,4-dimethyladipic acid is arbitrary, as the steric relations between these compounds and glyceraldehyde have not yet been determined (cf Part IX (2)).



IV V VI

(+)-2,3-dimethylheneicosanoic acid, $[M]_D^{23} + 66°$

## Experimental[1]

(+)-*1-Bromo-2,3-dimethylheneicosane* (III) derived from (—)-3,4-dimethyladipic acid.

(+)-3,4-Dimethyldocosanoic acid of $[M]_D^{25} + 19.7°$ (10 g) was dissolved in 99.5 % ethanol (200 ml), and a solution of potassium hydroxide (1.73 g) in 50 ml of 99.5 % ethanol added. A solution of silver nitrate (5.06 g) in 99.5 % ethanol (250 ml) was slowly added to the solution of the potassium salt under vigorous stirring. The precipitated silver salt was filtered off, washed with water and with methanol on the filter, and dried overnight at a temperature of 60° (in the dark). The slightly brownish dry salt (12.66 g) was finely powdered, and the final drying carried out *in vacuo* at a temperature of 90°. Powdered dry salt (12.66 g) was suspended in dry carbon tetrachloride (25 ml), and dry bromine added drop-wise to the refluxing mixture until the bromine colour persisted. The mixture was allowed to cool to room temperature, ether and water added, precipitated silver bromide removed, and the ether layer separated from the aqueous layer. The ether solution was washed with 5 % sodium carbonate solution in order to remove unchanged acid. Ethanol had to be added to suppress the strong tendency to formation of emulsion. After washing with water, the solution was dried with sodium sulphate and the solvents were distilled off. Crystallization of the residue from ethanol gave 5.62 g of slightly yellow crystals melting at 32–33°. After two crystallizations from acetone the *bromide* (III) was obtained as colourless lustrous plates melting at 33.5–34.0°. On slow cooling solidification usually occurred, at about 26°, to a beautifully crystalline solid. Rapid cooling produced a low-melting modification of m. p. 15.8–16.1°.

Long X-ray spacing (pressed specimen from acetone) 22.0 Å.

*Optical rotation* $\alpha_D^{24} + 0.34°$ (chloroform; *l*, 2; *c*, 2.82)
$[\alpha]_D^{24} + 6.0°$ $[M]_D^{24} + 24°$

| | | |
|---|---|---|
| *Anal.:* | Calcd for $C_{23}H_{47}Br$ (403.5) | Br, 19.81 % |
| | Found | Br, 19.70 % |

(+)-*2,3-Dimethylheneicosanoic acid* (VI) derived from (—)-3,4-dimethyladipic acid.

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[1] Melting points, crystal spacings and optical rotations were determined as described in Part I (5).

The bromide just described (4 g), potassium acetate (12 g), glacial acetic acid (25 ml), and butyl acetate (25 ml), were refluxed for 3 days. To the mixture, which had turned brown, was added water and ether. The ether layer was separated and washed with 5 % sodium carbonate, followed by water. Sodium sulphate and a small amount of charcoal were added, and filtration of the mixture gave a colourless filtrate. The solvents were removed, and the residue analyzed for bromine: found, Br, 0.98 %. The crude acetate (IV) was hydrolyzed by adding a solution of potassium hydroxide (15 g) in water (10 ml) and ethanol (200 ml), and refluxing the mixture for 4 hours. Water was added, and the mixture extracted with ether without acidification. The ether solution was washed with water, dried with sodium sulphate, and the solvents distilled off, leaving a practically white residue.

The crude alcohol (V) thus obtained was without further purification oxidized with potassium permanganate (3.0 g) in acetone (400 ml, distilled from potassium permanganate). The solution was left at room temperature (22–23°) for 4 days. After this time practically all permanganate colour had disappeared. A solution of hydrogen peroxide containing sulphuric acid was added until all manganese dioxide had been dissolved. The mixture was extracted with ether, and the ether solution washed with water and dried with sodium sulphate. The ether was distilled off, and the crystalline residue dissolved in ethanol. A solution of barium hydroxide in methanol-ethanol was made up from 10 ml of 1-*N* solution in methanol, 15 ml of methanol and 15 ml of ethanol, and this solution added to the ethanol solution of the residue from the oxidation. The mixture was placed in the refrigerator for a few hours, and the precipitate then removed by filtration. The precipitate, containing the barium salt of the desired acid (VI), was dried *in vacuo*. In order to remove ether-soluble matter, the dry salt was suspended in boiling ether. After cooling the mixture to 0° in the refrigerator, the barium salt was filtered off and decomposed by dilute hydrochloric acid. The free acid was taken up in ether, and the residue obtained on distilling off the solvent from the washed and dried ether solution crystallized from light petroleum (b. p. 40–60°) in the cold, giving 0.79 g of white, microcrystalline material melting at 64–65°. Recrystallization from acetone at 0° gave 0.63 g of *dextrorotatory 2,3-dimethylheneicosanoic acid* (VI) as lustrous plates of m. p. 65.8–66.1°. The yield was 18 % of the theoretical, calculated on the bromide (III).

Long X-ray spacing (pressed specimen) 30.8 Å.

*Optical rotation* $\alpha_{\mathrm{D}}^{23} + 1.05^\circ$ (chloroform; *l*, 2; *c*, 2.83)
$[\alpha]_{\mathrm{D}}^{23} + 18.6^\circ$ $[\mathrm{M}]_{\mathrm{D}}^{23} + 66^\circ$

The optical rotation of a solution of the acid in chloroform (*c*, 2.83) was also measured for several wavelengths, using a photoelectric ultraviolet polarimeter (cf Part VII (6)). The results are given below.

$[\mathrm{M}]_{5893}^{20} + 65.6^\circ$; $[\mathrm{M}]_{5893}^{25} + 66.2^\circ$; $[\mathrm{M}]_{5461}^{25} + 78.9^\circ$
$[\mathrm{M}]_{4358}^{25} + 137^\circ$ $[\mathrm{M}]_{4046}^{25} + 167^\circ$ $[\mathrm{M}]_{3650}^{25} + 224^\circ$
$[\mathrm{M}]_{3131}^{25} + 364^\circ$.

| | | | |
|---|---|---|---|
| *Anal.:* | Calcd for $C_{23}H_{46}O_2$ (354.6) | C, 77.90; | H, 13.08 % |
| | Found | C, 77.62; | H, 13.07 % |

The *amide* of the 2,3-dimethylheneicosanoic acid just described was prepared via the acid chloride. Crystallization from methanol gave the amide in the form of lustrous plates of m. p. 118.1–118.3°. When a small nucleus was present (to prevent supercooling), solidification began at 118.0° with the formation of long thin prisms. Remelting took place at 118.2–118.3°.

Long X-ray spacing (pressed specimen) 32.0 Å, calculated from a very good photograph.

*Anal.:* Calcd for $C_{23}H_{47}ON$ (353.6) N, 3.96 %
Found N, 3.87 %

(+)-*1-Bromo-2,3-dimethylheneicosane* (VIII) derived from *meso*-3,4-dimethyladipic acid.

This bromide was prepared from (+)-3,4-dimethyldocosanoic acid of $[M]_D^{24}$ + + 31.3° in the same manner as described for the stereoisomer (III). After crystallization from ethanol the bromide, which was obtained in a yield of 66 % of the theoretical, melted at 31–33°. After further crystallization from acetone the *bromide* was obtained in the form of small lustrous plates of m. p. 33.3—34.0°. Rapid cooling produced an unstable form melting below 26°. Immediately after this form had partially melted, the higher melting modification began to crystallize.

Long X-ray spacing (pressed specimen from acetone) 28.9 Å.

*Optical rotation* $\alpha_D^{23}$ + 0.92° (chloroform; *l*, 1 (micro tube; *c*, 13.6)
$[\alpha]_D^{23}$ + 6.8° $[M]_D^{23}$ + 27°

*Anal.:* Calcd for $C_{23}H_{47}Br$ (403.5) Br, 19.81 %
Found Br, 19.60 %

*Attempts to prepare the 2,3-dimethylheneicosanoic acid derived from* meso-*3,4-dimethyladipic acid.*

The bromide (VIII) was converted into alcohol acetate and free alcohol, and the alcohol oxidized, exactly as described for the stereoisomer. The product (0.49 g from 2.5 g of bromide) after crystallization from glacial acetic acid formed lustrous plates which melted over the temperature range 56° to 63°. Previously melted material behaved in the same manner, and the diffuse m. p. was not changed on further recrystallization. The material gave quite good X-ray diffraction patterns at room temperature, with a long spacing of 32.8 Å. In order to see whether the melting behaviour was possibly caused by polymorphic changes, a specimen was investigated in the high-temperature recording X-ray camera (7). However, no change in crystal structure on heating could be observed, the diffraction pattern disappearing in a continuous manner over the temperature range for melting. The optical rotation was $\alpha_D^{24}$ + 0.06° (chloroform; *l*, 1; *c*, 3.86).

*Anal.:* Calcd for $C_{23}H_{46}O_2$ (354.6) C, 77.90; H, 13.08 %
Calcd for $C_{23}H_{46}O_3$ (372.6) C, 74.14; H, 12.44 %
Found C, 74.10; H, 12.34 %

The *amide* of the above acid was prepared via the acid chloride and recrystallized from methanol. The amide was obtained as small lustrous plates which melted at 108.9–109.2°, unchanged after previous melting.

Long X-ray spacing (pressed specimen) 34.6 Å.

| | | |
|---|---|---|
| *Anal.:* | Calcd for $C_{23}H_{47}ON$ (353.6) | N, 3.96 % |
| | Calcd for $C_{23}H_{47}O_2N$ (371.6) | N, 3.77 % |
| | Found | N, 3.66 % |

The author is indebted to Mr. L. AHLQUIST for assistance in the synthetic work, and to Miss A. RENMAN and Mr. I. ALPEROWICZ for the analyses.

## SUMMARY

(+)-3,4-Dimethyldocosanoic acid derived from (—)-3,4-dimethyladipic acid has been degraded to the corresponding (+)-2,3-dimethylheneicosanoic acid. The silver salt of the 3,4-dimethylsubstituted acid is converted by HUNSDIECKER'S method into (+)-1-bromo-2,3-dimethylheneicosane. The bromide is converted into the corresponding alcohol, and the latter oxidized to the 2,3-dimethylsubstituted acid.

The dextrorotatory 2,3-dimethylheneicosanoic acid derived from (—)-3,4-dimethyladipic acid has the highest molecular rotation ($M_D$ + 66°) of the optically active aliphatic monocarboxylic acids so far synthesized.

Attempts to carry out the same type of degradation on (+)-3,4-dimethyldocosanoic acid derived from *meso*-3,4-dimethyladipic acid did not give the expected product. The compound obtained gave the correct analytical figures for a $C_{23}$ hydroxyacid, and it appears that in this case the oxidation of the intermediate alcohol leads preferentially to a 2-hydroxy-2,3-dimethylheneicosanoic acid with a partially or completely racemized α-carbon atom.

Institute of Medical Chemistry, University of Uppsala.

REFERENCES: 1. **Ställberg-Stenhagen, S.,** Arkiv för Kemi *1,* 153 (1949). — 2. *Idem, ibid., 3,* 249 (1951). — 3. **Hunsdiecker, H.,** and **Hunsdiecker, Cl.,** Berichte *75,* 291 (1942). — 4. **Meyer, R.,** Ann. *219,* 234 (1883). — 5. **Ställberg-Stenhagen, S.,** Arkiv för Kemi, Min. o. Geol. *23 A,* No. 15 (1946). — 6. *Idem,* Arkiv för Kemi *2,* 431 (1950). — 7. **Stenhagen, E.,** to be published shortly.

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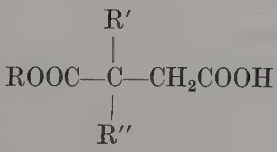
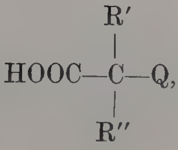
# Optically active higher aliphatic compounds. XI

## The synthesis of (−)-2-methyl-2-ethyleicosanoic acid

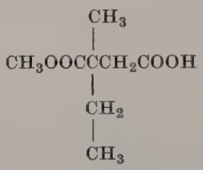
By Stina Ställberg-Stenhagen

Very few examples of optically active mono-carboxylic acids with an asymmetric quaternary α-carbon atom are recorded in the literature. The only case of a purely aliphatic acid of this type appears to be that of (+)-methylethylisobutylacetic acid recently described by Doering and Wiberg (1).

In connexion with the work on optically active long chain compounds described in earlier parts of this series, it was of interest also to study the properties of some optically active long chain acids having a quaternary α-carbon atom. The most convenient starting material for the synthesis of such acids appeared to be the readily available α, α-dialkylsuccinic acids. Birch and Robinson (2) have attempted to synthesize some inactive acids with a quaternary α-carbon atom by lengthening the chain of the appropriate asymmetric half-esters of α, α-dialkylsuccinic acids by the Robinson-Robinson method. Owing to the large difference in the rate of hydrolysis of the primary and tertiary ester end of esters of these acids (3), the preparation of the asymmetric half-ester with the ester group at the tertiary end is not difficult. It appeared possible either to resolve this half-ester or to prepare the active half-ester from previously resolved α, α-dialkylsuccinic acid, and then lengthen the chain at the free carboxyl end of the half-ester by some suitable method, *e.g.* mixed electrolysis of the sodium salt of the half-ester with the sodium salt of a monocarboxylic acid. In view of previous experiences of mixed electrolyses involving optically active half-esters (4), no trouble due to rearrangement of the ester groups was expected. Once the optically active half-ester of the structure  $\mathrm{ROOC{-}\overset{\displaystyle R'}{\underset{\displaystyle R''}{C}}{-}CH_2COOH}$ has been prepared, the series of optically active acids of the type  $\mathrm{HOOC{-}\overset{\displaystyle R'}{\underset{\displaystyle R''}{C}}{-}Q}$, where Q represents an alkyl group, become readily accessible.

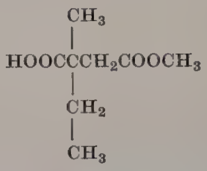
The synthesis of the laevorotatory enantiomorph of 2-methyl-2-ethyleicosanoic acid by the method outlined above, starting from α-methyl-α-ethylsuccinic acid, will be described.

The asymmetric methyl half-ester with the structure (I) was prepared by partial hydrolysis of the dimethyl ester, in a manner analogous to that described by BONE, SUDBOROUGH and SPRANKLING (5) (cf also FARMER and KRACOVSKI (6)) for the asymmetric mono-ester of α, α-dimethylsuccinic acid. Owing to the slow hydrolysis of the tertiary ester end, the mono-ester (I) is obtained in practically pure form (m.p. 34–35°) directly after distillation, the yield being about 90 % of the theoretical. The m.p. of the recrystallized mono-ester (I) was 35.8–36.3°.



$$CH_3OOC\overset{CH_3}{\underset{\underset{CH_3}{CH_2}}{C}}CH_2COOH$$

I



$$HOOC\overset{CH_3}{\underset{\underset{CH_3}{CH_2}}{C}}CH_2COOCH_3$$

II

For comparison, the second asymmetric mono-ester (II) was prepared by the action of sodium methoxide on the anhydride. The mono-ester (II) was obtained as a beautifully crystalline compound with m.p. 71.9–72.4°.

The mono-ester (I) gave a well-crystallizing quinine salt. Repeated crystallization of this salt from acetone-water (3 : 7 by volume) gave the pure salt of the laevorotatory enantiomorph. The *active mono-ester* (I), $[\alpha]_D^{23}$—11.50° (homogeneous), $[M]_D^{23}$—20.0°, has so far not been obtained in crystallized form. Hydrolysis of the active half-ester gave (—)-*2-methyl-2-ethylsuccinic acid* ((—)-α-methyl-α-ethylsuccinic acid), m.p. 64.6–65.4°, $[\alpha]_D^{22}$—5.9° (chloroform, *c*, 7.45). (—)-*Methyl 2-methyl-2-ethylsuccinate* prepared by esterification of the laevorotatory acid had $[\alpha]_D^{23}$—8.87° (homogeneous).

The lengthening of the chain of the laevorotatory half-ester (I) was carried out by mixed electrolysis, as outlined in the Chart.

The steric connections between α-methyl-α-ethylsuccinic acid and methylsuccinic acid have recently been determined by J. PORATH.[1] The method of quasi-racemic compounds was used and the determination performed by two independent routes (via ethylsuccinic acid and via α-methyl-α-*iso*propylsuccinic acid and *iso*propylsuccinic acid respectively), the result being that acids with the same configuration have the same sign of rotation for sodium light. It is assumed that the larger substituents at the α-carbon atoms correspond to each other, *i.e.* that the *methyl* group of methylsuccinic acid corresponds to the *ethyl* group in α-methyl-α-ethylsuccinic acid. It follows from this work that (—)-2-methyl-2-ethyleicosanoic acid is sterically related to L-glyceraldehyde. The formulae in the Chart are written in the manner employed in the FISCHER convention.

(—)-*Methyl 2-methyl-2-ethyleicosanoate* (V) was obtained in a yield of 25 % of the theoretical. The ester, which has an optical rotation $[\alpha]_D^{23}$—3.76° (homogeneous), $[M]_D^{23}$—13.9°, is dimorphous, with a stable form melting at 25.4–25.5° and an unstable form melting at 14.6–14.7°. Hydrolysis of (V) gave

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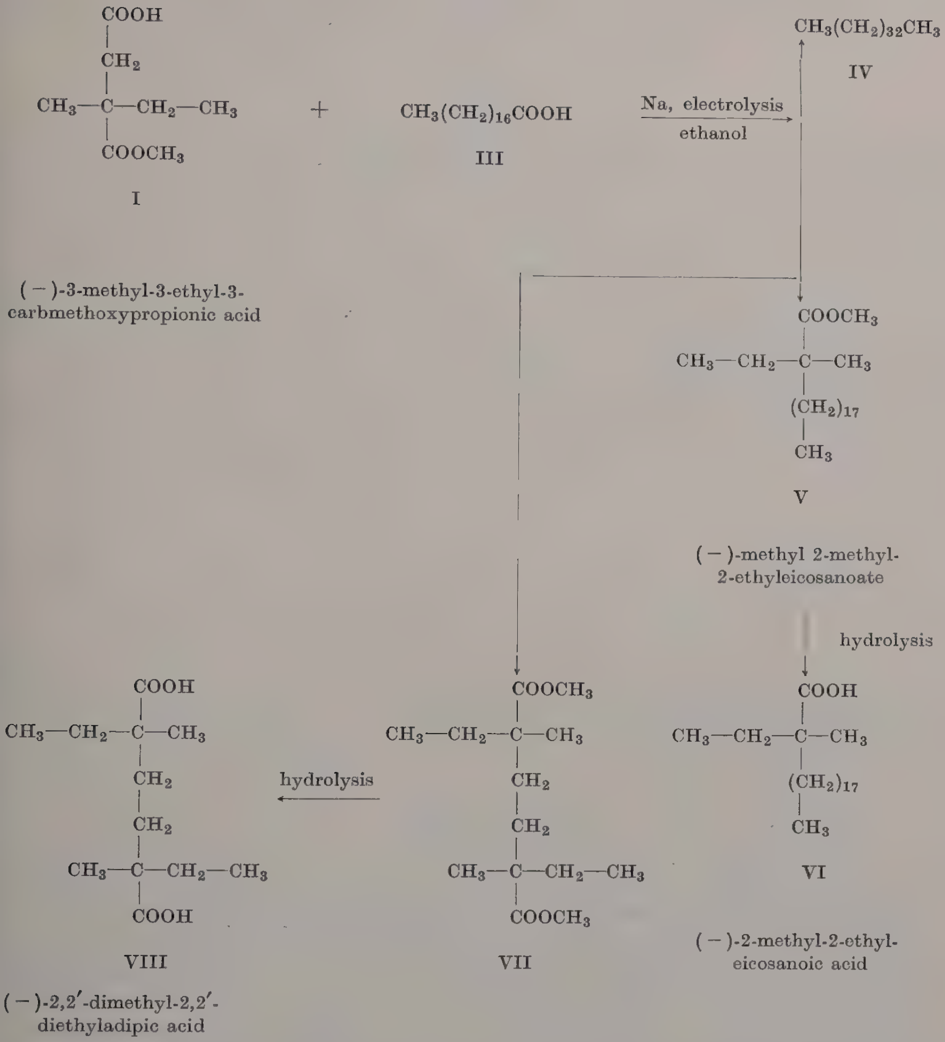
[1] Personal communication. In the course of publication in the *Arkiv för Kemi.*

**Chart**


COOH
CH₂
CH₃—C—CH₂—CH₃
COOCH₃
I
(−)-3-methyl-3-ethyl-3-carbmethoxypropionic acid
+
CH₃(CH₂)₁₆COOH
III
Na, electrolysis
ethanol
CH₃(CH₂)₃₂CH₃
IV
COOCH₃
CH₃—CH₂—C—CH₃
(CH₂)₁₇
CH₃
V
(−)-methyl 2-methyl-2-ethyleicosanoate
hydrolysis
COOH
CH₃—CH₂—C—CH₃
(CH₂)₁₇
CH₃
VI
(−)-2-methyl-2-ethyl-eicosanoic acid
COOCH₃
CH₃—CH₂—C—CH₃
CH₂
CH₂
CH₃—C—CH₂—CH₃
COOCH₃
VII
hydrolysis
COOH
CH₃—CH₂—C—CH₃
CH₂
CH₂
CH₃—C—CH₂—CH₃
COOH
VIII
(−)-2,2′-dimethyl-2,2′-diethyladipic acid

(—)-*2-methyl-2-ethyleicosanoic acid* (VI). This acid melts at 54.4–54.7° and has an optical rotation $[\alpha]_D^{23}$—4.28° (chloroform; *c*, 12,86), $[M]_D^{23}$—15.2°. The corresponding *amide* can exist in at least two crystalline modifications. A stable high-melting form melts at 58.3–58.7°, and a very unstable low-melting form which can be obtained on suitable cooling of the molten amide was found to melt at as low as 39°.

An unavoidable by-product from the electrolysis is the *laevorotatory methyl ester* (VII) of (—)-*2,2′-dimethyl-2,2′-diethyladipic acid* ((—)-α, α′-dimethyl-α, α′-diethyladipic acid) (VIII). The acid melts at 130–131° and has an optical rotation $[\alpha]_D^{25}$—6.5° (chloroform; *c*, 10.1), $[M]_D^{25}$—15.0°. The ester (VII) shows a rotation $[\alpha]_D^{23}$—13.4° (homogeneous), $[M]_D^{23}$—34.6°.

The rotatory dispersion for several of the optically active compounds has been determined, and the data are collected in Table I.

*Tab. I*

Molecular rotations at 25°

| Compound | Wavelength, Å | | | | | |
|---|---|---|---|---|---|---|
| | 3 131 | 3 650 | 4 046 | 4 358 | 5 461 | 5 893 |
| (−)-α-Methyl-α-ethylsuccinic acid (chloroform; *c*, 7.45) | | −14.9 | −15.5 | −14.6 | −10.7 | −9.5 |
| (−)-Methyl α-methyl-α-ethylsuccinate (homogeneous)[1] | | −50.3 | −39.5 | −33.1 | −19.5 | −16.7 |
| (−)-3-Methyl-3-ethyl-3-carbmethoxypropionic acid (homogeneous)[1] | −89 | −61.2 | −47.6 | −39.9 | −23.8 | −20.0 |
| (−)-Methyl 2-methyl-2-ethyleicosanoate (chloroform; *c*, 8.64) | −60 | −43.5 | −33.9 | −28.6 | −17.0 | −14.1 |
| (−)-2-Methyl-2-ethyleicosanoic acid (chloroform; *c*, 7.4) | −66 | −44.3 | −34.5 | −29.0 | −17.4 | −14.9 |
| (−)-α, α′-Dimethyl-α, α′-diethyladipic acid (chloroform; *c*, 10) | −50 | −40.7 | −32.6 | −27.8 | −17.0 | −15.0 |
| (−)-Methyl α, α′-dimethyl-α, α′-diethyladipate (homogeneous)[1] | | −101.9 | −80.1 | −67.5 | −41.1 | −34.6 |

## Experimental[2]

DL-*α-Methyl-α-ethylsuccinic acid* was prepared by the method of SMITH and HORWITZ (8). The crude acid was converted directly into the dimethyl ester, b.p. 71–72°, 3 mm, by means of methanol and sulphuric acid.

DL-*3-Methyl-3-ethyl-3-carbmethoxypropionic acid* (I). DL-Methyl α-methyl-α-ethylsuccinate (61.5 g), potassium hydroxide (21 g), water (21 ml) and methanol (250 ml) were kept at a temperature of 50° overnight. Water was added and the alkaline solution extracted with ether in order to remove unreacted dimethyl ester. Evaporation of the ether gave only a very small quantity of dimethyl ester. The alkaline solution was then acidified and extracted with ether. The ether solution was dried with sodium sulphate, the solvent distilled off, and the residue distilled under reduced pressure. The asymmetrical monomethyl ester (I) distilled at 96–97°, 1 mm. Yield 52 g or 91 % of the theoretical. The ester, which was obtained in the form of a colourless oil, solidified when kept in the refrigerator to a crystalline solid of m.p. 34–35°. After two crystallizations from light petroleum (b.p. 40–60°) at 0°, the half-ester (I) melted at 35.8–36.3°.

---

[1] At 23°.

[2] Melting points, crystal spacings, and optical rotations were measured as described in Parts I (7) and VII (9).

| | | |
|---|---|---|
| *Anal.*: Calcd for $C_8H_{14}O_4$ (174.2) | C, 55.16; | H, 8.10 % |
| Found | C, 55.10; | H, 8.15 % |

DL-*2-Methyl-2-ethyl-3-carbmethoxypropionic acid* (II). The isomeric asymmetrical half-ester (II) was prepared by dissolving DL-α-methyl-α-ethylsuccinic acid anhydride (20 g, obtained from the acid by the action of excess acetic anhydride) in a solution of sodium (3.24 g) in dry methanol (60 ml), and leaving the solution at room temperature for half an hour. Water was added and the solution acidified with dilute hydrochloric acid. Extraction with ether, and distillation of the residue after the ether solution had been dried and the solvent removed, gave 20.1 g of material boiling at 93–94°, 0.2 mm. The solidified material melted at 47–55°, and evidently consisted of a mixture of the two isomeric esters. Two recrystallizations from light petroleum (b.p. 40–60°) gave 10 g of the beautifully crystalline monomethyl ester (II). The melting point of this half-ester was 71.9–72.4°.

| | | |
|---|---|---|
| *Anal.*: Calcd for $C_8H_{14}O_4$ (174.2) | C, 55.16; | H, 8.10 % |
| Found | C, 54.93; | H, 8.27 % |

(—)-*3-Methyl-3-ethyl-3-carbmethoxypropionic acid* (I). Quinine (217 g) was dissolved in hot acetone (750 ml) and the solution filtered hot. Low-melting DL-monomethyl ester (I) (100 g) was added, followed by water (1 700 ml), and the solution left at room temperature overnight. The crystalline material from two similar crystallizations was combined, and the wet crystals (299 g) dissolved in 2 000 ml of 30 % acetone. The resulting crystalline material was recrystallized from 1 100 ml of 30 % acetone. The salt now obtained weighed 141 g. Part of the salt (15 g) was decomposed and the half-ester distilled, giving 2.55 g, b.p. 88°, 0.5 mm, $\alpha_{\mathrm{D}}^{23}$ —11.44° (homogeneous; $l$, 1). The main portion was further crystallized from 900 ml of 30 % acetone. Recovered half-ester now had a rotation $\alpha_{\mathrm{D}}^{23}$ —12.12° (hom., $l$, 1). The rotation increased to $\alpha_{\mathrm{D}}^{23}$ —12.46° after five crystallizations, and finally attained a value of $\alpha_{\mathrm{D}}^{23}$ —12.55° after the sixth crystallization.

$d_4^{23}$ 1.091.

| | | |
|---|---|---|
| *Optical rotation* | $\alpha_{\mathrm{D}}^{23}$ —12.55° | (homogeneous; $l$, 1) |
| | $[\alpha]_{\mathrm{D}}^{23}$ —11.50° | $[M]_{\mathrm{D}}^{23}$ —20.03° |

| | | |
|---|---|---|
| *Anal.*: Calcd for $C_8H_{14}O_4$ (174.2) | C, 55.16; | H, 8.10 % |
| Found | C, 55.20; | H, 8.15 % |

(—)-*α-Methyl-α-ethylsuccinic acid.* The active methyl ester just described was hydrolyzed and the free acid crystallized from water-concentrated hydrochloric acid (1:1). The acid formed transparent stout crystals melting at 64–65°. After recrystallization from light petroleum (b.p. 40–60°) containing a small amount of benzene, the laevorotatory acid formed thick prisms with m.p. 64.6–65.4°. The m.p. was unchanged after previous melting.

*Optical rotation* $\alpha_{\mathrm{D}}^{22}$—0.88° (chloroform; *l*, 2; *c*, 7.45)
$[\alpha]_{\mathrm{D}}^{22}$—5.9° $[\mathrm{M}]_{\mathrm{D}}^{22}$—9.5°

*Anal.*: Calcd for $C_7H_{12}O_4$ (160.2) C, 52.49; H, 7.55 %
Found C, 52.55; H, 7.63 %

(—)-*Methyl α-methyl-α-ethylsuccinate* was prepared from the acid just described and diazomethane in ether solution. The ester distilled at 72°, 3 mm.
$d_4^{23}$ 1.037.

*Optical rotation* $\alpha_{\mathrm{D}}^{23}$—1.84° (homogeneous; *l*, 0.2 (micro tube)
$[\alpha]_{\mathrm{D}}^{23}$—8.87° $[\mathrm{M}]_{\mathrm{D}}^{23}$—16.7°

*Anal.*: Calcd for $C_9H_{16}O_4$ (188.2) C, 57.43; H, 8.57 %
Found C, 57.60; H, 8.51 %

(—)-*Methyl 2-methyl-2-ethyleicosanoate* (V). The electrolysis was carried out as described in Part IX (9), with 11 g of active monomethyl ester (V), 17.9 g of stearic acid and 0.4 g of sodium in 300 ml of ethanol. The half-ester and the stearic acid were added in two portions. After the hydrocarbon (*n*-tetratriacontane) and remaining sodium stearate had been removed as previously described (7, 10), the residue was distilled *in vacuo*. The following fractions were collected.

| | | | | |
|---|---|---|---|---|
| I | up to 83° (mainly between 74 and 79°) 0.2 mm | 4.34 g | $\alpha_{\mathrm{D}}^{23}$—8.52° | (hom.; *l*, 1) |
| II | 83–146°, 0.2 mm | 0.8 g | | |
| III | 146–151°, 0.2 mm | 0.73 g | $\alpha_{\mathrm{D}}^{23}$—3.02° | ( » » ») |
| IV | 151–153°, 0.2 mm | 5.85 g | $\alpha_{\mathrm{D}}^{23}$—3.24° | ( » » ») |

Fraction IV consists of the desired methyl ester. The ester was obtained as a colourless oil, which on keeping at room temperature solidified to a beautifully crystalline solid melting at 24.6–25.0°. Crystallization from methanol gave the ester in the form of large thin plates with a beautiful lustre. The melting point was 25.4–25.5°. Rapid cooling under running water produced an unstable low-melting modification of m.p. 14.6–14.7°.
$d_4^{23}$ 0.862.
Long X-ray spacing (higher melting form) 27.4 Å.

*Optical rotation* $\alpha_{\mathrm{D}}^{23}$—3.24° (homogeneous; *l*, 1)
$[\alpha]_{\mathrm{D}}^{23}$—3.76° $[\mathrm{M}]_{\mathrm{D}}^{23}$—13.9°

*Anal.*: Calcd for $C_{24}H_{48}O_2$ (368.6) C, 78.19; H, 13.13 %
Found C, 78.36; H, 13.16 %

(—)-*2-Methyl-2-ethyleicosanoic acid* (VI) was prepared by alkaline hydrolysis of the methyl ester. After crystallization from light petroleum (b.p. 40–60°) the acid formed lustrous plates of m.p. 54.4–54.7°. Recrystallization from light petroleum gave the acid in the form of very beautiful thin lustrous plates, but the melting point was unchanged. The same m.p. was also found for previously melted material.
Long X-ray spacing (pressed specimen) 32.1 Å.

*Optical rotation* $\alpha_D^{23} - 1.10°$ (chloroform; $l$, 2; $c$, 12.86)
$[\alpha]_D^{23} - 4.28°$ $[M]_D^{23} - 15.2°$

*Anal.*: Calcd for $C_{23}H_{46}O_2$ (354.6) C, 77.90; H, 13.08 %
Found C, 77.89; H, 13.02 %

The *amide* of the laevorotatory acid was prepared via the acid chloride. After crystallization from methanol the *amide* formed lustrous plates melting at 53–54°. The crystals changed their appearance on standing, and the m.p. rose to 58.4–58.7°. If the specimen was taken out of the melting-point bath and allowed to solidify an unstable modification of m.p. 39° was produced. As soon as this melted, the higher melting form of m.p. 58.3–58.7° began to crystallize.

The X-ray diffraction pattern of a specimen freshly crystallized from methanol showed the presence of two crystalline forms, one of which corresponded to the high melting form. On slow heating, the other form present was transformed into the modification melting at 58.3–58.7°, and only the latter form was present in specimens that had been kept at room temperature for a long period of time. The X-ray diffraction patterns of the amide showed a large number of lines, but no series of $00l$ reflections could be recognized with certainty.

*Anal.*: Calcd for $C_{23}H_{47}ON$ (353.6) N, 3.96 %
Found N, 4.01 %

(—)-*Methyl 2,2′-dimethyl-2,2′-diethyladipate* (VII). Fraction I in the distillation of the electrolysis products (cf above) consists mainly of the ester (VII), together with small amounts of n-heptadecane and heptadecene-1 formed as by-products in the electrolysis. Fraction I was redistilled and the fraction distilling at 86–87°, 0.5 mm, collected. The analytical data for carbon and hydrogen indicated, however, that appreciable amounts of the hydrocarbons were still present. A pure specimen of the ester (VII) was therefore prepared from the acid (VIII) described below and diazomethane in ether solution. The pure ester distilled at 85–87°, 0.6 mm.
$d_4^{23}$ 0.983.

*Optical rotation* $\alpha_D^{23} - 6.59°$ (homogeneous; $l$, 0.5)
$[\alpha]_D^{23} - 13.4°$ $[M]_D^{23} - 34.6°$

*Anal.*: Calcd for $C_{14}H_{26}O_4$ (258.4) C, 65.08; H, 10.14 %
Found C, 65.00; H, 10.20 %

(—)-*2,2′-Dimethyl-2,2′-diethyladipic acid* (VIII). Hydrolysis of the crude methyl ester mentioned above, followed by crystallization of the crude acid from benzene, gave the *acid* (VIII) in the form of small transparent stout prisms melting at 130–131°. The m.p. was unchanged after further crystallization from benzene, and previously melted material also showed the same melting point.

*Optical rotation* $\alpha_D^{25}-0.66°$ (chloroform; *l*, 1; *c*, 10.1)
$[\alpha]_D^{25}-6.5°$ $[M]_D^{25}-15.0°$

*Anal.*: Calcd for $C_{12}H_{22}O_4$ (230.3) C, 62.58; H, 9.63 %
Found C, 62.52; H, 9.53 %

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## SUMMARY

The two asymmetric methyl half-esters of α-methyl-α-ethylsuccinic acid have been prepared. Both mono-esters are solid at room temperature, and the ester with a free primary carboxyl group is the lower melting one. The laevorotatory enantiomorph of the latter half-ester has been prepared via the quinine salt. Hydrolysis of the optically active half-ester gives (—)-α-methyl-α-ethylsuccinic acid.

Mixed electrolysis of the sodium salt of the optically active mono-ester and sodium stearate gave the methyl ester of (—)-2-methyl-2-ethyleicosanoic acid, which on hydrolysis gave the corresponding optically active long chain monocarboxylic acid with a quaternary α-carbon atom.

(—)-α, α′-Dimethyl-α, α′-diethyladipic acid has been prepared from the corresponding methyl ester obtained as by-product in the electrolysis.

The rotatory dispersion of several of the optically active compounds prepared has been measured over the near ultraviolet and the visible region.

REFERENCES. 1. **Doering, W. von E.**, and **Wiberg, K. B.**, J. Amer. Chem. Soc. *72*, 2608 (1950). — 2. **Birch, A. J.**, and **Robinson, Sir R.**, J. Chem. Soc. *1942*, 488. — 3. Cf **Meyer, H.**, Analyse und Konstitutionsermittlung organischer Verbindungen, 6th Ed., Vienna 1938, p. 481. — 4. **Ställberg-Stenhagen, S.**, Arkiv för Kemi *2*, 95 (1950). — **Bone, A. B.**, **Sudborough, J. T.** and **Sprankling, C. H. G.**, J. Chem. Soc. *85*, 534 (1904). — 6. **Farmer, E. H.** and **Kracovski, J.**, J. Chem. Soc. *1926*, 2318. — 7. **Ställberg-Stenhagen, S.**, Arkiv för Kemi, Min. o. Geol. *23 A*, No. 15 (1946). — 8. **Smith, P. A. S.** and **Horwitz, J. P.**, J. Amer. Chem. Soc. *71*, 3418 (1949). — 9. **Ställberg-Stenhagen, S.**, Arkiv för Kemi *2*, 431 (1950). — 10. *Idem, ibid.*, *3*, 249 (1951).

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# The stability of cyano-acids in water solution

By SIGVARD WIDEQVIST

In water solution at room temperature the cyano-acids show very different properties. This may be exemplified by the homologous series of phenyl-cyano-substituted fatty acids having the general formula $C_6H_5—CH(CN)—(CH_2)_n—COOH$.

Two members of this series have been previously investigated by the author (1, 2, 3), namely β-phenyl-β-cyanopropionic acid and γ-phenyl-γ-cyanobutyric acid. It was found that β-phenyl-β-cyanopropionic acid rapidly hydrolyzed in water solution even at room temperature and was converted into phenylsuccinamic acid. The amic acid was then further hydrolyzed to ammonium hydrogen phenyl-succinate. The γ-phenyl-γ-cyanobutyric acid, however, was quite stable under the same conditions.

Quantitative measurements on the rate of hydrolysis of β-phenyl-β-cyanopropionic acid were recently reported by the author (3).

In the present paper the investigation of the series of ω-phenyl-ω-cyano-acids is extended also to α-, δ-, and ε-acids.

The first member, phenylcyanoacetic acid, is, like its next homologue, not stable in water solution. It is, however, not hydrolyzed but decarboxylizes giving benzyl cyanide. In boiling solution the decarboxylation is almost instantaneous. HADLEY (4) reported that its cupric salt is vigorously decomposed even at room temperature.

The higher homologues, δ-phenyl-δ-cyanovaleric acid and ε-phenyl-ε-cyanocaproic acid, are not hydrolyzed in water.

In a previous investigation (2) it was found that β-phenyl-β-cyano*iso*butyric acid and β-phenyl-β-cyanopivalic acid were also hydrolyzed in water solution. β-Phenyl-α-cyanopropionic acid, however, was neither hydrolyzed nor decarboxylized.

It seems thus that only β-cyano-acids are hydrolyzed by water. This was confirmed in further experiments, and it was also found that β-cyano-acids are always spontaneously hydrolyzed in water solution. In a control experiment with α-phenyl-γ-cyanobutyric acid no hydrolysis was found.

It is very remarkable that the hydrolysis takes place with great ease in the case of acids which contain tertiary or aromatic bound cyano-groups, *e.g.* β-cyano*iso*valeric acid, β-phenyl-β-cyanobutyric acid and o-cyanobenzoic acid. The m- and p-cyanobenzoic acids are not hydrolyzed by water (5).

Cyano-groups on unsaturated carbon atoms are generally difficult to hydrolyze. Thus α-cyanocinnamic acid cannot be converted into benzalmalonic acid. The cyano-group is resistant to hydrolysis by acids and remains unaffected when the substance is dissolved in concentrated sulfuric acid or fuming hydrochloric acid (LAPWORTH and McRAE (6)). The isomeric β-cyanocinnamic acid, however, is extremly easily hydrolyzed by water.

It seems that the rate of hydrolysis increases with the number of substituents in the molecule. In fact, β-cyanopropionic acid, whose cyano-group is quite unprotected, is most slowly hydrolyzed.

In the present investigation the influences of the substituents on the rate of hydrolysis and the strength of the acids are studied.

The hydrolysis of the β-cyano-acids is catalyzed by the hydrogen ions coming from the acids themselves when they dissociate. There is, however, always a spontaneous, uncatalyzed reaction between the acids and the water.

The velocity of hydrolysis of the β-cyano-acids was determined by conductivity measurements.

As recently reported by the author (3) the following expression is obtained for the rate constant of the hydrolysis:

$$k = -\frac{(2-\alpha)(\psi-\alpha)}{(1-\alpha)^2 \varkappa_0} \frac{d\varkappa}{dt}. \tag{1}$$

This equation was derived for the hydrolysis of β-phenyl-β-cyanopropionic acid. All other β-cyano-acids, however, follow the same scheme. This implies that only the undissociated cyano-acids are hydrolyzed and that the hydrolysis is partly catalyzed by hydrogen ions and partly uncatalyzed. Thus we have

$$k = k_1 + k_2[\mathrm{H}^+]. \tag{2}$$

The symbols in equ. (1) and (2) have the following meaning:

$t$ = time in minutes
$k_1$ = rate constant of the uncatalyzed reaction
$k_2$ = rate constant of the hydrogen ion catalyzed reaction
$\varkappa_0$ = specific conductivity at zero time
$\psi = K/(K - K')$, where $K$ = the disssociation constant of the cyano-acid and $K'$ that of the corresponding amic acid.

The following additional symbols may also be introduced:

$c$ = concentration of the cyano-acid at zero time
$h$ = hydrogen ion concentration.

To determine the quantity $\psi$ for a certain cyano-acid, one does not necessarily require the knowledge of the dissociation constant of the corresponding amic acid, as $\psi$ can be estimated from other acids with the same number of substituents.

For β-cyanopropionic acid we get $\psi = 1.413$ (IVES and SAMES (7), JEFFERY and VOGEL (8)). When one substituent is introduced into β-cyanopropionic acid we get $\psi$ about 1.3. Thus we have $\psi$ for

| | |
|---|---|
| α-Phenyl-β-cyanopropionic acid . . . | 1.335 |
| β-Phenyl-β-cyanopropionic acid . . . | 1.302 |
| β-Cyanobutyric acid . . . . . . . . | 1.305. |

This relation also holds good for o-cyanobenzoic acid which gives $\psi = 1.318$.

For acids with two substituents (β-cyano*iso*valeric acid) we obtain $\psi = 1.173$ and for three substituents (β-phenyl-β-cyanopivalic acid. WIDEQVIST (2)) $\psi = 1.164$.

All conductivity measurements were performed at 25° C. Readings were taken at regular intervals over a period of from one to fifteen hours depending on the velocity of hydrolysis. From the readings the quantities $\varkappa_0$ and $d\varkappa/dt$ were calculated by the method of least squares. The velocity constants $k$, $k_1$, and $k_2$ were calculated with the same assumptions and in the same manner as described for β-phenyl-β-cyanopropionic acid. (WIDEQVIST (3)).

## Experimental

### Preparation of materials

The preparation of the cyano-acids has already been described (2, 5, 9, 10). Two of the amic acids which have not previously been described by the author are α-phenylsuccinamic acid and β, β-dimethylsuccinamic acid. α-Phenylsuccinamic acid was prepared by dissolving α-phenyl-β-cyanopropionic acid in conc. sulfuric acid. The solution was poured on crushed ice, when the amic acid separated. It was recrystallized from hot water. M.p. 145°. ANSCHÜTZ (11) gives the same melting point. β, β-Dimethylsuccinamic acid was obtained when β-cyano*iso*valeric acid was exposed to moist air for some time. It was recrystallized form hot acetone. M. p. 122–124°.

### The dissociation constants of the amic acids

The dissociation constants of β-methylsuccinamic acid, β, β-dimethylsuccinamic acid and α-phenylsuccinamic acid were determined. The results are shown in tables 1, 2, and 3.

*Table 1*

β-Methylsuccinamic acid. $\Lambda_0 = 378$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\alpha$ | $K \cdot 10^5$ |
|---|---|---|---|
| 3.266 | 32.27 | 0.08538 | 2.60 |
| 6.438 | 23.13 | 0.06118 | 2.57 |

Mean value $2.59 \cdot 10^{-5}$.

*Table 2*

β, β-Dimethylsuccinamic acid. $\Lambda_0 = 369$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\alpha$ | $K \cdot 10^5$ |
|---|---|---|---|
| 1.469 | 33.97 | 0.09206 | 1.37 |
| 3.223 | 23.05 | 0.06248 | 1.34 |
| 9.879 | 13.41 | 0.03634 | 1.35 |

Mean value $1.36 \cdot 10^{-5}$.

*Table 3*

α-Phenylsuccinamic acid. $\Lambda_0 = 368$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\alpha$ | $K \cdot 10^5$ |
|---|---|---|---|
| 1.305 | 85.39 | 0.2327 | 9.21 |
| 2.540 | 63.68 | 0.1730 | 9.19 |

Mean value $9.20 \cdot 10^{-5}$.

### The properties of the nonhydrolyzing cyano-acids

Phenylcyanoacetic acid is, like other α-cyano-acids, not hydrolyzed in water solution. The presence of the phenyl- and the cyano-group on the same carbon atom as the carboxy-group makes this group unstable. The acid thus decarboxylizes, giving benzyl cyanide. The decarboxylation causes a rapid fall in the conductivity of the acid. The degree of ionization of phenylcyanoacetic acid is unusually high. For that reason its thermodynamic dissociation constant was determined. Because of the instability of the acid, it was impossible to get its alkali salts free of carbonates. The dissociation constant was therefore directly calculated from the measurements on the free acid using IVES' formula (12). The results are shown in table 4.

*Table 4*

Thermodynamic dissociation constant of phenylcyanoacetic acid.
$\Lambda_0 = 375.4$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\Lambda_x$ | $\alpha$ | $K_c \cdot 10^2$ | $K_0 \cdot 10^2$ |
|---|---|---|---|---|---|
| 1.054 | 346.2 | 370.8 | 0.9337 | 1.39 | 1.29 |
| 2.043 | 328.0 | 369.2 | 0.8884 | 1.45 | 1.31 |
| 3.047 | 314.6 | 367.9 | 0.8551 | 1.52 | 1.35 |
| 4.092 | 300.1 | 367.0 | 0.8176 | 1.50 | 1.31 |

Mean value $1.31 \cdot 10^{-2}$.

α-Phenyl-γ-cyanobutyric acid is not hydrolyzed in water. Its dissociation constant is shown in table 5.

*Table 5*

α-Phenyl-γ-cyanobutyric acid. $\Lambda_0 = 368$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\alpha$ | $K \cdot 10^4$ |
|---|---|---|---|
| 1.110 | 94.00 | 0.2554 | 1.17 |
| 2.365 | 73.71 | 0.2003 | 1.19 |
| 3.832 | 59.24 | 0.1610 | 1.18 |
| 9.937 | 38.21 | 0.1038 | 1.20 |

Mean value $1.19 \cdot 10^{-4}$.

δ-Phenyl-δ-cyanovaleric acid and ε-phenyl-ε-cyanocaproic acid are also stable in water solution. They are very slightly soluble. A saturated solution of the δ-acid at 25° C is about 0.007-N and of the ε-acid 0.005-N. The dissociation constants of the acids are not very different from those of the corresponding amic acids. For ε-phenyl-ε-cyanocaproic acid $K = 1.78 \cdot 10^{-5}$ and for the amic acid $K = 1.64 \cdot 10^{-5}$. This implies that it is almost impossible to use the conductivity method to determine whether the acids hydrolyze or not. For that reason samples of the acids were shaken with water at room temperature for six weeks, after which time the acids were quite unchanged, and no ammonium ions could be detected in their solutions with Nessler's reagent. The dissociation constants are shown in tables 6 and 7.

*Table 6*

δ-Phenyl-δ-cyanovaleric acid. $\Lambda_0 = 367.4$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\alpha$ | $K \cdot 10^5$ |
|---|---|---|---|
| 1.533 | 42.43 | 0.1155 | 2.31 |
| 2.615 | 33.13 | 0.0875 | 2.31 |
| 5.223 | 23.55 | 0.0641 | 2.29 |

Mean value $2.30 \cdot 10^{-5}$.

*Table 7*

ε-Phenyl-ε-cyanocaproic acid. $\Lambda_0 = 367$.

| $c \cdot 10^3$ | $\Lambda_c$ | $\alpha$ | $K \cdot 10^5$ |
|---|---|---|---|
| 0.942 | 47.06 | 0.1282 | 1.78 |
| 1.823 | 34.90 | 0.0951 | 1.82 |
| 3.624 | 24.62 | 0.0671 | 1.75 |

Mean value $1.78 \cdot 10^{-5}$.

## The hydrolyzing cyano-acids

The velocity of hydrolysis was measured on the following cyano-acids:

- β-Cyanopropionic acid
- β-Cyanobutyric acid
- β-Cyano*iso*valeric acid
- α, α-Dimethyl-β-cyanobutyric acid
- α-Phenyl-β-cyanopropionic acid
- γ-Phenyl-β-cyanobutyric acid
- β-Phenyl-β-cyanobutyric acid
- o-Cyanobenzoic acid.

The measurements also involved the determination of their dissociation constants. The constant of o-cyanobenzoic acid was, however, reported previously (5).

It was found that the unsubstituted β-cyanopropionic acid was most slowly hydrolyzed. The branched acids hydrolyzed more rapidly. The effect of the hydrogen ions was greatest on the acids containing tertiary cyano-groups. The results of the measurements are shown in tables 8–15. The quantity $d\varkappa/dt$ is negative, but the minus-sign is omitted from the tables.

### *Table 8*

β-Cyanopropionic acid. $\Lambda_0 = 380.6$; $\psi = 1.413$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^4$ | $k \cdot 10^5$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 1.884 | 0.15246 | 0.175 | 0.2126 | 4.01 | 1.08 | 3.97 | 3.98 |
| 4.178 | 0.23442 | 0.292 | 0.1474 | 6.16 | 1.07 | 4.02 | 4.05 |
| 11.56 | 0.40421 | 0.561 | 0.0919 | 10.62 | 1.07 | 4.25 | 4.22 |
| 18.63 | 0.51733 | 0.736 | 0.0730 | 13.59 | 1.07 | 4.28 | 4.32 |
| 51.60 | 0.87223 | 1.423 | 0.0444 | 22.92 | 1.07 | 4.78 | 4.66 |
| 101.26 | 1.2341 | 2.102 | 0.0320 | 32.43 | 1.07 | 4.94 | 5.01 |

$K = 1.07 \cdot 10^{-4}$; $k_1 = 3.83 \cdot 10^{-5}$; $k_2 = 3.64 \cdot 10^{-3}$.

### *Table 9*

β-Cyanobutyric acid. $\Lambda_0 = 379$; $\psi = 1.305$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^4$ | $k \cdot 10^4$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 3.962 | 0.23141 | 1.117 | 0.1541 | 6.12 | 1.11 | 1.43 | 1.42 |
| 6.082 | 0.29437 | 1.467 | 0.1277 | 7.77 | 1.14 | 1.44 | 1.48 |
| 12.66 | 0.42703 | 2.489 | 0.0890 | 11.27 | 1.10 | 1.63 | 1.60 |
| 25.63 | 0.60707 | 3.859 | 0.0625 | 16.02 | 1.07 | 1.74 | 1.75 |

$K = 1.11 \cdot 10^{-4}$; $k_1 = 1.22 \cdot 10^{-4}$; $k_2 = 3.35 \cdot 10^{-2}$.

### *Table 10*

β-Cyano*iso*valeric acid. $\Lambda_0 = 370.5$; $\psi = 1.173$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^5$ | $k \cdot 10^4$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 0.890 | 0.08951 | 1.748 | 0.2714 | 2.42 | 9.00 | 5.73 | 5.75 |
| 1.764 | 0.13277 | 3.317 | 0.2032 | 3.58 | 9.14 | (6.85) | 6.07 |
| 3.321 | 0.18885 | 4.682 | 0.1535 | 5.10 | 9.24 | 6.51 | 6.48 |
| 5.564 | 0.24910 | 6.797 | 0.1208 | 6.72 | 9.24 | 6.98 | 6.93 |
| 11.64 | 0.36783 | 11.49 | 0.0853 | 9.93 | 9.26 | 7.71 | 7.81 |
| 22.19 | 0.51341 | 18.69 | 0.0624 | 13.84 | 9.22 | 8.91 | 8.88 |

$K = 9.18 \cdot 10^{-5}$; $k_1 = 5.09 \cdot 10^{-4}$; $k_2 = 2.74 \cdot 10^{-1}$.

### *Table 11*

α, α-Dimethyl-β-cyanobutyric acid. $\Lambda_0 = 369.5$; $\psi = 1.164$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^4$ | $k \cdot 10^3$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 1.327 | 0.12062 | 4.606 | 0.2640 | 3.26 | 1.07 | 1.08 | 1.09 |
| 2.633 | 0.17745 | 7.895 | 0.1824 | 4.80 | 1.07 | 1.19 | 1.18 |
| 3.478 | 0.20691 | 9.757 | 0.1610 | 5.60 | 1.08 | 1.24 | 1.23 |
| 4.708 | 0.24387 | 12.02 | 0.1402 | 6.60 | 1.08 | 1.27 | 1.28 |

$K = 1.07 \cdot 10^{-4}$; $k_1 = 9.03 \cdot 10^{-4}$; $k_2 = 5.75 \cdot 10^{-1}$.

*Table 12*

α-Phenyl-β-cyanopropionic acid. $\Lambda_0 = 369$; $\psi = 1.335$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^4$ | $k \cdot 10^4$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 1.202 | 0.18636 | 0.492 | 0.4201 | 5.05 | 3.66 | 1.14 | 1.15 |
| 2.037 | 0.25702 | 0.788 | 0.3420 | 6.97 | 3.62 | 1.17 | 1.16 |
| 4.145 | 0.39335 | 1.369 | 0.2572 | 10.66 | 3.69 | 1.18 | 1.18 |
| 8.001 | 0.57070 | 2.168 | 0.1932 | 15.47 | 3.70 | 1.20 | 1.21 |

$K = 3.67 \cdot 10^{-4}$; $k_1 = 1.11 \cdot 10^{-4}$; $k_2 = 6.08 \cdot 10^{-3}$.

*Table 13*

γ-Phenyl-β-cyanobutyric acid. $\Lambda_0 = 368$; $\psi = 1.305$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^4$ | $k \cdot 10^4$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 0.557 | 0.07574 | 0.171 | 0.3694 | 2.06 | 1.21 | 0.865 | 0.889 |
| 1.382 | 0.13047 | 0.393 | 0.2566 | 3.55 | 1.22 | 0.995 | 0.955 |
| 2.483 | 0.18222 | — | 0.1995 | — | 1.23 | — | — |
| 3.368 | 0.21610 | 0.743 | 0.1744 | 5.87 | 1.24 | 1.04 | 1.06 |
| 6.649 | 0.31091 | 1.258 | 0.1270 | 8.45 | 1.23 | 1.17 | 1.17 |

$K = 1.23 \cdot 10^{-4}$; $k_1 = 7.99 \cdot 10^{-5}$; $k_2 = 4.41 \cdot 10^{-2}$.

*Table 14*

β-Phenyl-β-cyanobutyric acid. $\Lambda_0 = 368$; $\psi = 1.173$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $K \cdot 10^4$ | $k \cdot 10^4$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | found | calcd. |
| 0.544 | 0.06945 | 0.301 | 0.3470 | 1.89 | 1.00 | 1.39 | 1.37 |
| 1.485 | 0.12647 | 1.057 | 0.2314 | 3.44 | 1.04 | 2.36 | 2.33 |
| 2.317 | 0.16173 | 1.717 | 0.1897 | 4.39 | 1.03 | 2.88 | 2.93 |
| 4.695 | 0.24024 | 3.791 | 0.1390 | 6.53 | 1.05 | 4.29 | 4.25 |

$K = 1.03 \cdot 10^{-4}$; $k_1 = 1.96 \cdot 10^{-5}$; $k_2 = 6.22 \cdot 10^{-1}$.

*Table 15*

o-Cyanobenzoic acid. $\Lambda_0 = 377$; $\psi = 1.318$.

| $c \cdot 10^3$ | $1000\,\varkappa_0$ | $\frac{d\varkappa}{dt} \cdot 10^8$ | $\alpha$ | $h \cdot 10^4$ | $k \cdot 10^3$ | |
|---|---|---|---|---|---|---|
| | | | | | found | calcd. |
| 0.598 | 0.14723 | 2.217 | 0.6531 | 3.91 | 1.12 | 1.17 |
| 1.164 | 0.23617 | 7.860 | 0.5382 | 6.26 | 1.78 | 1.70 |
| 2.556 | 0.39391 | 24.85 | 0.4088 | 10.45 | 2.61 | 2.63 |

$k_1 = 2.94 \cdot 10^{-4}$; $k_2 = 2.24$.

As well as the acids above, two other cyano-acids were examined, namely β, β-diphenyl-β-cyanopropionic acid (prepared from diphenylacetonitrile and ethyl bromoacetate by the method of SALMON-LEGAGNEUR (13)) and β-cyanocinnamic acid. These acids are not suitable for exact measurements in pure water, as they are very slightly soluble. The first acid is almost insoluble. It was, however, found that they hydrolyzed in water like other β-cyano-acids. The velocity of hydrolysis for β-cyanocinnamic acid is exceptionally high. This can be illustrated by the following data:

$$c = 0.001223;\ \varkappa_0 = 0.25120;\ d\varkappa/dt = 7.00 \cdot 10^{-7}.$$

With $\Lambda_0 = 369$ we get $h = 6.81 \cdot 10^{-4}$, $K = 8.55 \cdot 10^{-4}$, and $k = 3.84 \cdot 10^{-2}$. For the corresponding saturated acid, β-phenyl-β-cyanopropionic acid $K = 1.62 \cdot 10^{-4}$ and $k = 1.18 \cdot 10^{-4}$ at the same pH. Thus β-cyanocinnamic acid hydrolyzes more than 300 times faster than β-phenyl-β-cyanopropionic acid.

## SUMMARY

The stability in water solution of a number of cyano-acids has been investigated. In agreement with previous observations it was found that the β-cyano-acids were rapidly hydrolyzed to succinamic acids. The velocity of hydrolysis increased with the number of substituents. The catalytic effect of hydrogen ions was greatest on acids containing tertiary cyano-groups.

Acids having the cyano-group in α-, γ-, δ, or ε-position were not hydrolyzed.

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REFERENCES: (1) **Wideqvist,** Arkiv Kemi, Mineral. Geol. *14 B,* No. 19 (1940). — (2) ——, Svensk Kem. Tid. *54,* 34 (1942). — (3) ——, Arkiv Kemi, *3,* No. 19 (1951). — (4) **Hadley,** J. Am. Chem. Soc. *34,* 924 (1912) — (5) **Wideqvist,** Arkiv Kemi *2,* 383 (1950). — (6) **Lapworth** and **McRae,** J. Chem. Soc. *121,* 1702 (1922). — (7) **Ives** and **Sames,** J. Chem. Soc. *1943,* 513. — (8) **Jeffery** and **Vogel,** J. Chem. Soc. *1934,* 1101. — (9) **Wideqvist,** Arkiv Kemi, Mineral. Geol. *26 A,* No. 16 (1948) — (10) ——, Arkiv Kemi, *3,* No. 7 (1951). — (11) **Anschütz,** Ann. *354,* 123 (1907). — (12) **Ives,** J. Chem. Soc. *1933,* 731. — (13) **Salmon-Legagneur,** Compt. rend. *208,* 1507 (1939).

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# Some aspects of the cyano-substituted carboxylic acids

By Sigvard Wideqvist

## Introduction

For a long time cyano-acids and numerous cyano-acids known in the form of esters have attracted the interest of investigators. The main importance of these cyano-acids and cyano-esters is as intermediate products in the preparation of dicarboxylic acids.

The study of the free cyano-acids, however, has not attracted much attention. Most of the cyano-acids, described in the literature, are the easily prepared α-cyano-acids.

The cyano-acids are often unsatisfactorily or erroneously characterized. A curious example is α-cyanopropionic acid which Wanklyn and Cooper (11) believed to have prepared in 1879 by the oxidation of wool with potassium permanganate. They reported that the acid had the formula $C_2H_4(CN)COOH, 1\frac{1}{2}\,H_2O$ and decomposed at 140°. This substance was even up to the present time recorded in chemical handbooks as α-cyanopropionic acid, although Ramberg (12) as early as 1902 had prepared and described the true α-cyanopropionic acid.

Many other examples of errors and mistakes could be given. The purpose of the present investigation, the results of which are summarized in this paper, is, however, not to give a general revision of earlier investigations in this field, but only to describe a few representative types of cyano-acids. From these examples it is possible to draw conclusions concerning the preparation and properties of other cyano-acids having a similar constitution.

Most attention is paid to the β-cyano-acids, whose most remarkable property is their easy hydrolysis even in pure water.

## Methods of preparation

Cyano-acids may be prepared either directly, or with their esters as intermediate products. The esters are, in most cases, easily saponified by alcoholic potassium or sodium hydroxide. There is no risk of hydrolysis of the cyano-group, when the saponification is performed at room temperature. The use of acidic hydrolysing agents should be avoided.

The most important methods of preparation may be summarized as follows.

## A. The introduction of a cyano-group

Introduction of cyano-groups into carboxylic acids and esters may be carried out in the following ways.

1. By the action of potassium cyanide on alkali salts of α-halogenated carboxylic acids:

$$R\text{–}CHBr\text{–}COOK + KCN \rightarrow R\text{–}CH(CN)\text{–}COOK + KBr.$$

In this way cyanoacetic acid and α-cyanopropionic acid are prepared in good yields. (Ramberg (12), Hoffmann and Barbier (13), Wideqvist (3)). The use of halogeno-esters instead of the free acids is not advisable, as side reactions complicate the synthesis. (Zelinsky (14).)

2. When the cyano-group is to be introduced into a position other than the α-position, the halogeno-esters must be used:

$$Br\text{–}(CH_2)_n\text{–}COOC_2H_5 + KCN \rightarrow CN\text{–}(CH_2)_n\text{–}COOC_2H_5 + KBr.$$

(Ives and Sames (15)).

3. By decomposition of α-oximinodicarboxylic acids;

$$HOCO\text{–}(CH_2)_n\text{-}C(:NOH)\text{–}COOH \rightarrow HOCO\text{–}(CH_2)_n\text{–}CN + H_2O + CO_2.$$

(Cramer (16), Blaise and Gault (17)).

4. By the action of Chloramin T on α-aminodicarboxylic acids:

$$HOCO\text{–}(CH_2)_n\text{–}CH(NH_2)\text{–}COOH \rightarrow \left(HOCO\text{–}(CH_2)_n\text{–}CH(NCl_2)COOH\right) \rightarrow$$
$$\rightarrow HOCO\text{–}(CH_2)\text{–}CN + 2\ HCl + CO_2.$$

Dakin (18) prepared β-cyanopropionic acid in this way.

5. Be the action of potassium cyanide and water on alkylidenemalonic esters, which yields β-cyano-acids:

$$RR'C{=}C(COOC_2H_5)_2 + KCN + H_2O \rightarrow RR'C(CN)\text{–}CH_2\text{–}COOC_2H_5 + KHCO_3 + C_2H_5OH.$$

By this method, the following acids were prepared: β-phenyl-β-cyanopropionic acid (Bredt and Kallen (19), Anschütz (20), Wideqvist (21)), β-furyl-β-cyanopropionic acid (Sandelin (22)), β-cyanobutyric acid (Bredt and Kallen (19)) and β-cyanoisovaleric acid (Wideqvist (8)).

6. By Sandmeyer's method. In this way the o-, m-, and p-cyano-benzoic acids were prepared from the aminobenzoic acids. (Sandmeyer (23), Kattwinkel and Wolffenstein (24), Valby and Lucas (25), Wideqvist (7)).

## B. Carboxylation of nitriles

Nitriles are conveniently carbethoxylated by means of diethyl carbonate with the use of sodium, sodium ethoxide, sodamide etc. as condensing agents:

$$R–CH_2–CN + CO(OC_2H_5)_2 \xrightarrow{Na} R–CNa(CN)–COOC_2H_5 + C_2H_5OH + \tfrac{1}{2} H_2.$$

(HESSLER (26), WALLINGFORD, JONES, and HOMEYER (27), WIDEQVIST (4), PETTERSSON (28)).

## C. Condensation of nitriles with halogeno-esters

Certain nitriles, in form of alkali salts, are easily condensed with halogeno-esters, *e.g.* diphenylacetonitrile:

$$(C_6H_5)_2C(CN)Na + Br–CH_2–COOC_2H_5 \rightarrow (C_6H_5)_2C(CN)–CH_2–COOC_2H_5 + NaBr.$$

(SALMON-LEGAGNEUR (29)). Hydratroponitrile reacts in the same way (CRAWFORD (30), WIDEQVIST (8)).

## D. Condensation of α-cyano-esters with halogeno-esters

The reaction leads to cyanodicarboxylic esters which may be saponified to monoesters, which are then decarboxylized by heating, yielding esters of cyanomonocarboxylic acids. (PERKIN and LAPWORTH (31), WIDEQVIST (5)). As a special case, the reaction between ethyl phenylcyanoacetate and halogeno-esters, which leads to α-phenyl-α-cyanodicarboxylic esters, should be mentioned. When heated with alkali, these esters are saponified and decarboxylated in one operation, giving ω-phenyl-ω-cyano-acids in excellent yield. Other arylcyanoacetic esters react in the same way. (WIDEQVIST (1, 5, 8).

## E. Cyanoethylation of arylmalonic esters

Malonic esters add acrylonitrile forming [β-cyanoethyl]-malonic esters. When the malonic esters contain an aryl-group, they exhibit properties similar to the α-aryl-α-cyanodicarboxylic esters described above. When treated with alkali, they are saponified and partially decarboxylated in one operation giving α-aryl-γ-cyanobutyric acids in good yield. (WIDEQVIST (8)).

## F. Alkylation of cyanoacetic ester

1. By the action of alkyl halogenides on sodiocyanoacetic ester.
2. By condensation of aldehydes and ketones with cyanoacetic ester (KNOEVENAGEL (32), COPE, HOFMANN, WYCKOFF, and HARDENBERG (33), WIDEQVIST (34)). The resulting alkylidenecyanoacetic esters may by hydrogenated to give alkyl derivatives. This method of preparing alkylcyanoacetic esters is superior to the alkylation by alkyl halogenides. It gives better yield, and avoids dialkylation.

It is also possible to add Grignard reagents to alkylidenecyanoacetic esters (REIMER and KOHLER (35)). In the case of ketone derivatives the addition products are *tert*-alkylcyanoacetic esters. *tert*-Butylcyanoacetic acid was prepared by WIDEQVIST (6) in this way. This method has also been investigated by ALEXANDER, McCOLLUM, and PAUL (36), who were inspired by the author's work on the synthesis of *tert*-alkylmalonic esters (37).

## Difficulties in the preparation of cyano-acids

There are three main reactions which may obstruct a successful preparation of cyano-acids, namely decarboxylation, hydrolysis of the cyano-group and formation of cyclic imides.

### A. Decarboxylation

Decarboxylation limits the preparation of α-aryl-α-cyano-acids. Phenylcyanoacetic acid is unstable and decarboxylizes gradually in water solution even at room temperature. Alkylated phenylcyanoacetic acids are instantaneously decarboxylized to alkylphenylacetonitriles. In fact, this is the best method for the preparation of such nitriles in very pure state. Hydratroponitrile was prepared in this way by saponification of α-cyanohydratropic ester (WIDEQVIST (2)). On further hydrolysis, racemic hydratropic acid was obtained in crystalline form for the first time by FREDGA and WIDEQVIST (38). α-Cyanocarboxylic acids which do not contain aryl-groups in α-position are decarboxylized only at high temperature (HOFFMANN and BARBIER (13)).

### B. Hydrolysis of the cyano-group

The hydrolysis of the cyano-group is of importance only in the case of β-cyano-acids. These acids are always easily hydrolyzed to succinamic and succinic acids even in pure water. It is therefore essential that the β-cyano-acids be rapidly removed from their water solutions, when they have been liberated from their salts. Fortunately the succinamic acids are insoluble in alcohol-free ether which permits the isolation of the cyano-acids in pure state.

### C. Formation of imides

The formation of imides is a troublesome complication in the preparation of β- and γ-cyano-acids. Even SANDMEYER (23) failed in the preparation of o-cyanobenzoic acid because of the formation of phthalimide. BONE and PERKIN jr. (39) thought that they had prepared α, α-dimethyl-β-cyanobutyric acid by decarboxylation of trimethylcyanosuccinic acid. There is, however, no doubt that their product consisted of trimethylsuccinimide (WIDEQVIST (5)). BLAISE (40) tried to prepare α α-dimethyl-β-cyanopropionic acid in a similar way and recognized the reaction product as dimethylsuccinimide. The true α, α-dimethyl-β-cyanobutyric was prepared by the author (5) who found properties quite other than those reported by BONE and PERKIN jr. Like other β-cyano-acids, α, α-dimethyl-β-cyanobutyric acid was rapidly hydrolyzed in water solution, whereas the "acid" of BONE and PERKIN jr. required several hours' boiling with conc. hydrochloric acid. It is curious that BONE and PERKIN jr. did not detect their mistake. Some years later PERKIN jr. and LAPWORTH (31) investigated trimethylcyanoglutaric ester. This ester, on hydrolysis with alkali or conc. hydrochloric acid, yielded trimethylglutarimide which was very resistant to hydrolysis. For complete hydrolysis it was necessary to heat the imide in a sealed tube at 200° for five hours with conc. hydrochloric acid. Similar observations were made on ethyl γ-cyano-β, β-dimethylglutarate.

The imide formation of β- and γ-cyanoacids is a special case of the reaction between carboxylic acids and nitriles described by GAUTIER (41) and COLBY and DODGE (42): When a mixture of acetonitrile and acetic acid was heated in a sealed tube at 250° for seven hours, diacetamide was formed.

In WHITMORE, "Organic Chemistry" (43) the following somewhat confusing sentence appears: "Aliphatic carboxylic acids add to nitriles to form diacidamides. This tendency is so great that reactions which would be expected to give γ-cyanopropionic (!) acid actually give succinimide by internal addition".

The apprehension concerning the β-cyano-acids is, however, over-estimated. The imide formation, in the case of β-cyano-acids, takes place only at elevated temperatures.

When a sample of β-cyanoisovaleric acid, m.p. 78–79°, was slowly distilled at atmospheric pressure, it was observed by the author that the distillate was an almost colorless oil which soon solidified. After recrystallization from water, the product had the m.p. 104–105°. For dimethylsuccinimide the m.p. 106° is reported by LEVY and ENGLÄNDER (44).

The statement of ANSCHÜTZ (20) that β-phenyl-β-cyanopropionic acid had been purified by vacuum distillation (B.p. 215–218°/10 mm) seems questionable. When pure β-phenyl-β-cyanopropionic acid was kept at 100° for some time, it was converted into a brownish, sticky mass which did not recrystallize, even when it was seeded with crystals of β-phenyl-β-cyanopropionic acid. It should be mentioned that ANSCHÜTZ, as well as BREDT and KALLEN, states that β-phenyl-β-cyanopropionic acid melts at 150°. The correct melting point is, however, 75° (WIDEQVIST (21)).

## The properties of the cyano-acids

### General remarks

The properties of the cyano-acids are mainly dependent on the position of the cyano-group. The more remarkable reactions of the cyano-acids, namely decarboxylation, hydrolysis and imide formation, are already described. Consequently we find, that α-cyano-acids may be decarboxylized and that the tendency for decarboxylation increases with the number of substituents on the α-carbon atom. All β-cyano-acids are unstable in water solution and hydrolyze to succinamic acids. The β- and γ-cyano-acids may be converted to imides by heating. Some concrete examples of physical properties of cyano-acids are given below.

## Aliphatic cyano-acids

The investigated aliphatic cyano-acids are listed below together with their melting points:

| Acid | M. p. |
|---|---|
| α-Cyanopropionic | 35.0° |
| β-Cyanopropionic | 49–50° |
| β-Cyanobutyric | oil |
| β-Cyanoisovaleric | 78–79° |
| α, α-Dimethyl-β-cyanobutyric | 112—113° |

α-Cyanopropionic acid is extremely hygroscopic and liquefies rapidly in the air. It is not quite stable, and when it was kept in a stoppered bottle for some time, it smelled strongly of hydrogen cyanide. β-Cyanobutyric acid is, when freshly prepared, a colorless oil which, however, darkens after a short time. The other β-cyano-acids seem to be stable when kept in desiccator over phosphorous pentoxide. Upon contact with moist air, however, they are rapidly converted into succinamic acids.

## The homologue series of ω-phenyl-ω-cyano-acids

The variation of the properties of the cyano-acids with increasing distance between the cyano-group and the carboxy-group may be illustrated by the homologue series of ω-phenyl-ω-cyano-acids.

There is a close parallelism in properties between the ω-phenyl-ω-cyano-acids and the corresponding phenyldicarboxylic acids. Thus phenylmalonic acid is easily decarboxylized like phenylcyanoacetic acid. In water solution it begins to decarboxylize at about 40° (Jakubowicz (45)). The cyano-acid decarboxylizes even at room temperature. Both series of acids (except phenylcyanoacetic acid) show alternating melting points as indicated in the table below.

| ω | Cyano-acid | Dicarboxylic acid |
|---|---|---|
| α | 91° | 151.5° |
| β | 75° | 167° |
| γ | 61° | 82–83° |
| δ | 75–76° | 132–133° |
| ε | 54–55° | 92–93° |

The solubility in water diminishes rapidly with increasing chain length. The α- and β-acids are moderately soluble, but exact measurements on the solubilities cannot be performed, as the acids decompose in water. The γ-acid liquefies under water and is therefore also unsuitable for measurements. Saturated solutions of the δ- and ε-acids contain 0.007 and 0.005 mole/l respectively at 25°. Unlike the other ω-phenyl-ω-cyano-acids, phenylcyanoacetic acid forms a monohydrate melting at 75°.

## The phenylcyanopropionic acids

The phenylcyanopropionic acids may also be regarded as derivatives of hydrocinnamic acid and hydratropic acid.

α-Cyanohydrocinnamic acid, m.p. 101°, is not hydrolyzed by water. When heated together with copper powder it decarboxylizes and is converted into β-phenylpropionitrile (BAKER and LAPWORTH (46)).

β-Cyanohydrocinnamic acid, m.p. 75°, hydrolyzes in water solution.

α-Cyanohydratropic acid is known only as the ethyl ester. The free acid decarboxylizes immediately yielding hydratroponitrile (WIDEQVIST (2)).

β-Cyanohydratropic acid, m.p. 97°, is like β-cyanohydrocinnamic acid hydrolyzed in water solution.

## The phenylcyanobutyric acids

Five phenylcyanobutyric acids were prepared, namely α-phenyl-γ-cyanobutyric acid, m.p. 89–90°, β-phenyl-β-cyanobutyric acid, m. p. 80–84°, γ-phenyl-β-cyanobutyric acid, m.p. 91–92°, γ-phenyl-γ-cyanobutyric acid, m.p. 61°, and β-phenyl-β-cyanoisobutyric acid, m.p. 99–100°.

The β-cyano-acids hydrolyzed in the usual manner. The γ-cyano-acids did not show any tendency toward spontaneous imide formation.

## The cyanobenzoic acids

All the three cyanobenzoic acids were investigated. It was found that o-cyanobenzoic acid has the same properties as β-cyano-acids. Thus it was easily hydrolyzed by water. As reported by other investigators, it formed phthalimide when heated over the melting point. The m- and p-cyanobenzoic acids were quite stable in water solution (WIDEQVIST (7)).

## The dissociation constants

The dissociation constants of the cyano-acids were determined at 25° using OSTWALD's dilution law, except in the case of phenylcyanoacetic acid. Because of this acid's unusual strength its thermodynamic dissociation constant was determined.

The dissociation constants are listed in the table below.

| References | Acid | $K \cdot 10^4$ |
|---|---|---|
| (3) | α-Cyanopropionic | 42.7 |
| (3) | β-Cyanopropionic | 1.07 |
| (10) | β-Cyanobutyric | 1.11 |
| (10) | β-Cyanoisovaleric | 0.918 |
| (10) | α, α-Dimethyl-β-cyanobutyric | 1.07 |
| (10) | Phenylcyanoacetic | 131 |
| (10) | α-Phenyl-β-cyanopropionic | 3.67 |
| (1) | β-Phenyl-α-cyanopropionic | 57.0 |
| (1) | β-Phenyl-β-cyanopropionic | 1.62 |

| References | Acid | $K \cdot 10^4$ |
|---|---|---|
| (10) | α-Phenyl-γ-cyanobutyric | 1.19 |
| (10) | β-Phenyl-β-cyanobutyric | 1.03 |
| (10) | γ-Phenyl-β-cyanobutyric | 1.23 |
| (1) | γ-Phenyl-γ-cyanobutyric | 0.394 |
| (1) | β-Phenyl-β-cyanoisobutyric | 2.00 |
| (10) | δ-Phenyl-δ-cyanovaleric | 0.230 |
| (1) | β-Phenyl-β-cyanopivalic | 1.12 |
| (10) | ε-Phenyl-ε-cyanocaproic | 0.178 |
| (7) | o-Cyanobenzoic | 7.29 |
| (47) | m-Cyanobenzoic | 2.3 |
| (25) | p-Cyanobenzoic | 3.09 |

## The hydrolysis of the β-cyano-acids

### Previous investigations

The property of the β-cyano-acids of being spontaneously hydrolyzed in pure water solution even at room temperature is remarkable. It is especially remarkable that also cyano-acids containing tertiary or aromatic bound cyano-groups are easily hydrolyzed. It is a common opinion that tertiary cyano-groups are difficult to hydrolyze. In GILMAN, "Organic Chemistry" (48) we read: "Like other addition reactions, the hydrolysis is very sensitive to changes in the nature of the substituent group R. Thus it is extremely difficult to hydrolyze tertiary aliphatic nitriles or di-ortho substituted aromatic nitriles."

It is curious that the easy hydrolysis of the β-cyano-acids was not detected previously, since at least two β-cyano-acids were conductometrically investigated before. These acids were o-cyanobenzoic acid and β-cyanopropionic acid. The o-cyanobenzoic acid was studied by SCHEIBER (49) who determined its dissociation constant and found that it decreased when the solution was diluted. It is, however, evident that he did not observe that the conductivity decreased with the time, and he states: "Da fremde Beimengungen, ebenso Verseifung zu Phthalaminsäure als ausgeschlossen gelten können, liegt es nahe, den Gang der Konstanten durch intramolekulare Veränderung zu erklären."

β-Cyanopropionic acid was investigated by IVES and SAMES (15). These authors carefully checked whether the acid hydrolyzed in water solution or not, and they report as follows: »In all cases one of the measured solutions was kept in the conductivity cell overnight in order to detect any solvolytic reaction which might have invalidated the treatment of the results. No such reaction was found in any case". It must, however, be said that it might be difficult to observe the hydrolysis on the β-cyanopropionic acid, as it hydrolyzes more slowly than the other cyano-acids. Furthermore IVES and SAMES worked with very dilute solutions, which made the observations far more difficult.

There are, however, two other acids which contain a cyano-group in β-position to the carboxy-group, namely thiocyanoacetic acid and α-thiocyanopropionic acid. The cyano-groups in these acids are very rapidly hydrolyzed. The hydrolysis of the thiocyanoacetic acid was observed by OSTWALD (50) who determined the dissociation constant of the acid. He made, however, no measurements on the velocity of the hydrolysis. The first measurements in this field were performed by FREDGA (51, 52) who investigated α-thiocyanopropionic acid and determined the velocity of hydrolysis by polarimetric measurements on optically active acid. He stated that only the undissociated thiocyano-acid hydrolyzes and that its anion is stable. This statement was later confirmed by WIDEQVIST (53) who determined the rate of hydrolysis by conductivity measurements. In the same way, the velocity of hydrolysis of thiocyanoacetic acid was determined.

No previous measurements on the hydrolysis of cyano-acids seem to have been made.

At last it should be mentioned that SANDELIN (22) observed that β-furyl-β-cyanopropionic acid was converted into β-furylsuccinamic acid, when its water solution was boiled.

## Reaction order for the hydrolysis

In two previous papers on the hydrolysis of β-cyano-acids (9, 10), it was stated that only the undissociated part of the cyano-acid hydrolyzes and, that the hydrolysis is partly uncatalyzed and partly catalyzed by hydrogen ions. Thus we obtain the following differential equation, when $[H_2O]$ is assumed to be constant.

$$dx/dt = (k_1 + k_2[H^+])(1-\alpha)(c-x) \tag{1}$$

where $(c-x)$ = the concentration of the cyano-acid, $\alpha$ the degree of ionization and $k_1$ and $k_2$ the velocity constants for the uncatalyzed and catalyzed reactions respectively.

Instead of the assumption above we may assume that both the undissociated cyano-acid and its anion are hydrolyzed, but at different rates, and that the hydrolysis is always catalyzed by hydrogen ions. In this case we get the following differential equation

$$dx/dt = [H^+][(k_3\alpha + k_2(1-\alpha)](c-x). \tag{2}$$

Equ. (2) may be rearranged in the following way: According to the dissociation equation, if $K$ = the dissociation constant of the cyano-acid, we obtain

$$[H^+]\alpha = K(1-\alpha)$$

On introducing this expression into equ. (2) we get

$$dx/dt = (Kk_3 + k_2[H^+])(1-\alpha)(c-x).$$

On putting $Kk_3 = k_1$ we obtain equ. (1). Thus equ. (1) and (2) are identical and it is impossible to choose between them. Equ. (1) is, however, to be preferred.

## The calculation of the velocity constants

When the cyano-acids hydrolyze, they are converted into amic acids, which are weaker than the cyano-acids. It is therefore possible to follow the hydrolysis by conductivity measurements, since the hydrolysis causes a decrease in conductivity with time.

For the velocity constant of the hydrolysis, the following expression is obtained (9)

$$k = -\frac{(2-\alpha)(\psi-\alpha)}{(1-\alpha)^2 \varkappa_0} \frac{d\varkappa}{dt} \tag{3}$$

where $k = k_1 + k_2[\mathrm{H}^+]$, $\varkappa_0$ = specific conductivity at zero time, $d\varkappa/dt$ = the change in conductivity at zero time, $\alpha$ = the degree of ionization of the cyano-acid. $\psi = K/(K-K')$, where $K$ and $K'$ = the dissociation constants of the cyano-acid and the corresponding amic acid respectively. This expression was derived by the author (9) assuming constant ion mobilities and the validity of OSTWALD's dilution law. It could therefore be expected that the value of $k$ would be false. This is, however, not the case. Equ. (3) has been tested for the hydrolysis of α-thiocyanopropionic acid. The rate constant found, using equ. (3), was in good agreement with that found by FREDGA (51) from polarimetric measurements.

It is, however, clear that $k$ is not exact, but a close approximation of the true value. If thermodynamic data are available and the influence of the ion concentration upon the ion mobilities is taken into account, we obtain

$$k_0 = -\frac{(2-\alpha)(\psi-\alpha)}{(1-\alpha)^2 \varkappa_0 \left(\frac{3}{2} - \Lambda_0/2\Lambda_x\right)} \frac{d\varkappa}{dt} \tag{3 a}$$

where $\Lambda_0$ is the equivalent conductivity of the cyano-acid at infinite dilution and $\Lambda_x$ the sum of the ion mobilities at the actual ion concentration.

The equ. (3) and (3 a) have been compared for β-phenyl-β-cyanopropionic acid. For this acid we have the thermodynamic dissociation constant

$$K_0 = 1.48 \cdot 10^{-4} \quad \text{and} \quad \Lambda_0 = 377.61$$

(unpublished measurements by the author). The values of $k$ and $k_0$ at different concentrations of the acid are compared below:

| $c \cdot 10^3$ | 1.298 | 4.768 | 4.916 | 7.710 | 9.668 | 15.64 | 19.71 |
|---|---|---|---|---|---|---|---|
| $k \cdot 10^4$ | 1.113 | 1.214 | 1.182 | 1.232 | 1.268 | 1.244 | 1.293 |
| $k_0 \cdot 10^4$ | 1.112 | 1.218 | 1.185 | 1.238 | 1.274 | 1.252 | 1.291 |

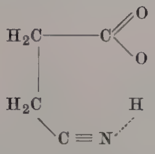
The differences between $k$ and $k_0$ lie within the limits of the experimental errors. It is thus quite unimportant whether equ. (3) or (3 a) is used.

## Some aspects of the hydrolysis of the β-cyano-acids

The rate constants for the hydrolysis of the investigated aliphatic cyano-acids are shown below.

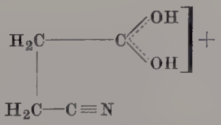
| Acid | $k_1$ | $k_2$ |
|---|---|---|
| β-Cyanopropionic | $3.83 \cdot 10^{-5}$ | $3.63 \cdot 20^{-3}$ |
| β-Cyanobutyric | $1.22 \cdot 10^{-4}$ | $3.35 \cdot 10^{-2}$ |
| β-Cyanoisovaleric | $5.09 \cdot 10^{-4}$ | $2.74 \cdot 10^{-1}$ |
| α, α-Dimethyl-β-cyanobutyric | $9.03 \cdot 10^{-4}$ | $5.75 \cdot 10^{-1}$ |

An inspection of the table shows that the rate constant $k_1$, for the spontaneous reaction, is lowest for the unsubstituted β-cyanopropionic acid and increasing with the number of substituents. It is obvious that the easy hydrolysis of the β-cyano-acids depends on the direct interaction between the cyano-group and the carboxy-group. It seems thus to be a close parallelism between the hydrolysis and the imide formation. The imides are, however, not intermediate products in the hydrolysis. When the imides are formed, they are fairly difficult to hydrolyze. But it is likely that both the hydrolysis of the cyano-group and the imide formation takes place over a common transition state containing a hydrogen bond:



Under these circumstances it is not surprising that the reaction velocity increases with the number of substituents. The influence of the substituents on the velocity of ring formation was investigated by **Hjelt** (54) and **Sebelius** (55) in the case of lactones. It was found that the velocity of lactone formation increased with the number of substituents. Further examples of the influence of substituents on ring formation are given by **Hückel** (56).

Even the velocity constant $k_2$, for the hydrogen catalyzed reaction, increases with the number of substituents. This is also in agreement with the theory of direct interaction between the cyano- and carboxy-group. The first stage in the reaction is presumably the addition of a proton to the carboxy-group, yielding the transition state (**Dewar** (57))



Because of the positive charge on the carboxy-group the chelation is facilitated, and by means of the hydrogen bond a proton can be transferred to the cyano-group. The cyano-group is then easily attacked by the water molecules.

Let us now consider the cyano-acids containing phenyl-groups. The following velocity constants were determined:

| Acid | $k_1$ | $k_2$ |
|---|---|---|
| α-Phenyl-β-cyanopropionic | $1.11 \cdot 10^{-4}$ | $6.08 \cdot 10^{-3}$ |
| β-Phenyl-β-cyanoproprionic | $1.10 \cdot 10^{-4}$ | $1.13 \cdot 10^{-2}$ |
| β-Phenyl-β-cyanobutyric | $1.96 \cdot 10^{-5}$ | $6.22 \cdot 10^{-1}$ |
| γ-Phenyl-β-cyanobutyric | $7.99 \cdot 10^{-5}$ | $4.41 \cdot 10^{-2}$ |
| o-Cyanobenzoic | $2.94 \cdot 10^{-4}$ | 2.24 |

The two phenylcyanopropionic acids have almost identical values for $k_1$. The effect of the phenyl group is thus independent of its position and differs only slightly from that of a methyl-group. The effect of the hydrogen ions on α-phenyl-β-cyanopropionic acid is half as great as on the β-phenyl-β-cyanopropionic acid.

In β-phenyl-β-cyanobutyric acid, which contains a tertiary cyano-group, the effect of the phenyl-group on the hydrolysis is great. This acid evidently affords an example of reaction hindrance. The spontaneous reaction is suppressed and the velocity reduced below that of β-cyanopropionic acid. The effect of the hydrogen ions is, however, more than twice as great as in the case of β-cyanoisovaleric acid. In γ-phenyl-β-cyanobutyric acid, however, the influence of the phenyl-group on the velocity of hydrolysis is slight. This is in good agreement with the observations of SEBELIUS (55) who states that the influence of substitutents in a side chain of the lactone ring is of secondary importance.

In o-cyanobenzoic acid the free rotation of the substituent groups is inhibited, and the groups are fixed in a position which is suitable for an interaction between the groups. It would thus be expected that o-cyanobenzoic acid would be very rapidly hydrolyzed. This is also the case. Although the spontaneous reaction is not remarkable rapid, the hydrogen ion catalyzed reaction is more rapid than for any other of the investigated cyano-acids.

It seems that the substituents influence the hydrolysis of the cyano-group in two ways: Partly by promoting the hydrolysis in the same manner as they promote imide formation, and partly by protecting the cyano-group. Because of the protecting effect the velocity of the spontaneous reaction may be reduced as in the case of β-phenyl-β-cyanobutyric acid. Some aspects on the hydrolysis of ordinary nitriles are given by MIGRDICHIAN (58).

## Appendix

Most of that which has been said about the β-cyano-acids is also valid for the corresponding succinamic acids. The amic acids are thus easily hydrolyzed in water solution, yielding acid ammonium salts of substituted succinic acids. When the conductivity of a succinamic acid is measured, it is found that the conductivity at first rapidly decreases, passes through a minimum and then rapidly increases. A typical run of measurements on phthalamic acid is recorded in the paper (7) by the author. Here the conductivity minimum is obtained after about 70 minutes. In the case of dimethyl-succinamic acid the minimum was passed after 5 minutes.

When the succinamic acids are heated, they lose water and are converted into their corresponding succinimides. This tendency is sometimes so great that the amic acid cannot be recrystallized in the usual manner. This is the case for phthalamic acid. When it is dissolved in a hot solvent, the imide separates from the solution.

## Acknowledgments

The present investigation has been carried out at the Institute of Chemistry, University of Uppsala, during the years 1940–1950. The work was, however, discontinued for some years during the war.
To Professor Arne Fredga I wish to express my sincere gratitude for his kind interest in my work and for many stimulating discussions. I also wish to express my thanks to Professor Gunnar Hägg for that time, when I worked at his department.

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REFERENCES: 1) **Wideqvist,** Svensk Kem. Tid. *54,* 34 (1942). — 2) ——, Svensk Kem. Tid. *55,* 125 (1943). — 3) ——, Arkiv Kemi, Mineral. Geol. *19 B,* No. 9 (1945). — 4) ——, Arkiv Kemi, Mineral. Geol. *24 B,* No. 14 (1947). — 5) ——, Arkiv Kemi, Mineral. Geol. *26 A,* No. 16 (1948). — 6) ——, Arkiv Kemi. *2,* 321 (1950). — 7) ——, Arkiv Kemi. *2,* 383 (1950). — 8) ——, Arkiv Kemi. *3,* 59 (1951). — 9) ——, Arkiv Kemi. *3,* 147 (1951). — 10) ——, Arkiv Kemi. *3,* No. 28 (1951). — 11) **Wanklyn** and **Cooper,** Phil. Mag. [5] *7,* 356 (1879). — 12) **Ramberg,** Studier i kemisk kinetik. Diss. Lund 1902, 40. — 13) **Hoffmann** and **Barbier,** Bull. Soc. Chim. Belg. *45,* 565 (1936). — 14) **Zelinsky,** Ber. *21,* 3160 (1888). — 15) **Ives** and **Sames,** J. Chem. Soc. *1943,* 513. — 16) **Cramer,** Ber. *24,* 1207 (1891). — 17) **Blaise** and **Gault,** Bull. Soc. Chim. [4] *9.* 457 (1911). — 18) **Dakin,** Biochem. Journ. *11,* 84 (1918). — 19) **Bredt** and **Kallen,** Ann. *293,* 343 (1896). — 20) **Anschütz,** Ann. *354,* 123 (1907). — 21) **Wideqvist,** Arkiv Kemi, Mineral. Geol. *14 B,* No. 19 (1940). — 22) **Sandelin,** Ber. *33,* 487 (1900). — 23) **Sandmeyer,** Ber. *18,* 1497 (1885). — 24) **Kattwinkel** and **Wolffenstein,** Ber. *34,* 2423 (1901). — 25) **Valby** and **Lucas,** J. Am. Chem. Soc. *51,* 2718 (1929). — 26) **Hessler,** Am. Chem. J. *32,* 127 (1904). — 27) **Wallingford, Jones,** and **Homeyer,** J. Am. Chem. Soc. *64,* 576 (1942). — 28) **Pettersson,** Acta Chem. Scand. *4,* 395 (1950). — 29) **Salmon-Legagneur,** Compt. rend. *208.* 1507 (1939). — 30) **Crawford,** J. Am. Chem. Soc. *56,* 139 (1934). — 31) **Perkin** and **Lapworth,** J. Chem. Soc. *75,* 48, 61 (1899). — 32) **Knoevenagel,** Ber. *37,* 4461 (1905). — 33) **Cope, Hofmann, Wyckoff,** and **Hardenberg,** J. Am. Chem. Soc. *63,* 3452 1941). — 34) **Wideqvist,** Acta Chem. Scand. *3,* 303 (1949). — 35) **Reimer** and **Kohler,** Am. Chem. J. *33,* 333 (1905). — 36) **Alexander, McCollum,** and **Paul,** J. Am. Chem. Soc. *72,* 4791 (1950). — 37) **Wideqvist,** Arkiv Kemi, Mineral. Geol. *23 B.* No. 4 (1946). — 38) **Fredga** and **Wideqvist,** Acta Chem. Scand. *1,* 860 (1947). — 39) **Bone** and **Perkin** jr., J. Chem. Soc. *67,* 416 (1895). — 40) **Blaise,** Bull. Soc. Chim. [3] *21,* 535 (1899). — 41) **Gautier,** Compt. rend. *67,* 1255 (1868). — 42) **Colby** and **Dodge,** Am. Chem. J. *13,* 1 (1891). — 43) **Whitmore,** Organic Chemistry, New York 1946, 501. — 44) **Levy** and **Engländer,** Ann. *242,* 205 (1887). — 45) **Jakubowicz,** Z. anorg. Ch. *121,* 123 (1922). — 46) **Baker** and **Lapworth,** J. Chem. Soc. *125,* 2333 (1924). — 47) **Kilpatrick** and **Eanes,** J. Am. Chem. Soc. *65,* 589 (1943). — 48) **Gilman,** Organic Chemistry, New York 1944, 660. — 49) **Scheiber,** Ber. *45,* 2401 (1912). — 50) **Ostwald,** Z. physik. Chem. *3,* 179 (1889). — 51) **Fredga,** J. pr. Chem. [2] *123,* 110 (1929). — 52) ——, Arkiv Kemi, Mineral. Geol. *13 A,* No. 5 (1938). — 53) **Wideqvist,** Svensk Kem. Tid. *55,* 284 (1943). — 54) **Hjelt,** Ber. *24,* 1237 (1891). — 55) **Sebelius,** Zur Kenntnis der Laktone, namentlich ihrer Hydrolyse, Diss. Lund 1927, 207, 222. — 56) **Hückel,** Theoretische Grundlagen der organischen Chemie II, Leipzig 1935, 257. — 57) **Dewar,** The Electronic Theory of Organic Chemistry, Oxford 1949. — 58) **Migrdichian,** The Chemistry of Organic Cyanogen Compounds, New York 1947, 37.

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Communicated 28 February 1951 by ARNE FREDGA

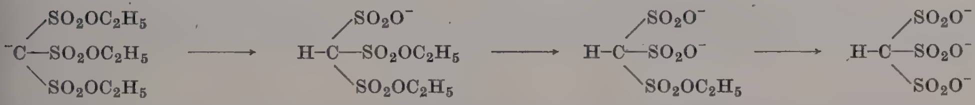
# On ethyl methanetrisulfonate and its hydrolysis

By EVALD SAMÉN

With 3 figures in the text

In previous papers an account is given of the preparation of methyl methanetrisulfonate (1) and the hydrolysis of the same ester (2). The analogous ethyl ester will be reported here. The ester reacts rapidly with bromine in several solvents. Like the methyl ester the aqueous solution of the ethyl ester will react so rapidly with bromine that under certain conditions the reaction is faster than the reaction between bromine and an azo indicator such as methyl orange. The methanetrisulfonic acid is very slowly brominated at room temperature. The plausible assumption is made that only the unhydrolyzed trisulfonic ester or its carbanion, but not the two acid esters will react immediately with bromine.

The disappearance of the ester can then be followed by removing samples of the reacting system from time to time and titrating them with a bromine solution. When the logarithm of the ester concentration is plotted against time the reaction is shown to be pseudounimolecular, in a solution where the hydronium ion concentration can be considered approximately constant. The velocity of the first stage of the reaction in the hydrolysis scheme



is however changed by varying the hydronium ion concentration. The rate of the hydrolysis appears to be proportional to this concentration.

For convenience, the following symbols will be introduced:

$t$ time from the start of the reaction (minutes)
$c_0$ the initial molar concentration of the ester
$c_x$ the actual molar concentration of the ester at time $t$
$c_h$ the hydronium ion concentration at time $t$
$k$ and $k_2$ rate constants of the first and second order reactions

If the velocity of the hydrolysis depends on the two concentration terms $c_x$ and $c_h$ the following equation is valid:

$$-\frac{d c_x}{d t} = k_2 c_x c_h .$$

On integration this becomes

$$k_2 = \frac{1}{\int_0^t c_h \, dt} \ln c_0 / c_x . \tag{1}$$

When the ethyl ester is brominated the hydrolysis products are acids and consequently, in aqueous solution only, is the hydronium ion concentration considerably increased. Here $c_h$ is an unknown function $f(t)$, because the second and the third hydrolysis constants are not known. The hydrolysis however can be followed by titration with alkali. On the reasonable assumption that the hydrolysis products are strong acids, the titration values obtained will then give $c_h$. This can be plotted against time $t$, and from the diagram, the area $\int_0^t c_h \, dt$ can be evaluated. $k_2$ is found using this integral in equ. (1). The ester itself appears to be a strong acid and therefore $f(0)$ is taken as equal to $c_0$.

## Experimental

**Preparation of ethyl methanetrisulfonate.** The ester is prepared by heating the water-free silver salt of methanetrisulfonic acid with ethyl iodide in benzene. 5.8 g (0.01 mole) of the silver salt and 5 g of ethyl iodide in 30 ml of benzene were refluxed gently for fourteen hours. The reaction product was practically pure. Yield, 2.4 g of ester.

Attempts to prepare the ester by boiling the silver salt with ethyl bromide instead of ethyl iodide were not successful.

The compound can be recrystallized from a mixture of benzene and petroleum ether. It separates in fine needles. M. P. 75° C. The ester is sparingly soluble in petroleum ether but easily soluble in acetone, alcohol, benzene, chloroform and ether. When the compound is kept in the open air, it gradually adsorbs water and liquefies.

*Anal.* Subs., 26.95 mg: 24.37 mg $CO_2$, 11.60 mg $H_2O$
26.17 mg: 9.38 ml of 0.0490 $N$ NaOH

| | | | | |
|---|---|---|---|---|
| $C_7H_{16}O_9S_3$ (340.4) | calc. | C 24.70 | H 4.74 | S 28.26 |
| | found | C 24.68 | H 4.82 | S 28.15 |

The hydrolysis in hot water is rapid. 36.27 mg of ester was dissolved in 15 ml of water and boiled for one minute. Neutralization required 6.48 ml of 0.0490 $N$ NaOH. Eq.-w. = 114.2. Calc. 113.5.

**Reaction of the ester with bromine.** If bromine water is added to an aqueous solution of the ethyl ester a white precipitate is formed immediately, but the product dissolves rapidly however. The compound itself and the clear solution too, liberate iodine from potassium iodide. After some time the solution no longer has this effect on potassium iodide. A solution of the ester in absolute ethyl alcohol will also react immediately with bromine. In acetone

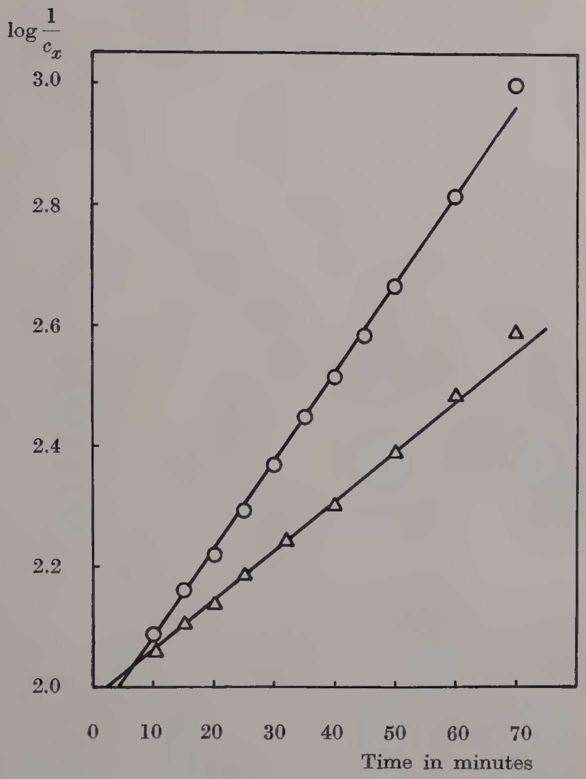


Fig. 1. Hydrolysis of ethyl methanetrisulfonate in 0.100 *N* HCl (○) and in 0.050 *N* HCl (△), both with $c_0 = 0.01$ *M*.

the analogous reaction is not so fast and in glacial acetic acid the reaction is rather slow. The bromine color however fades away instantly when a few drops water are added to the glacial acetic acid.

**Kinetic measurements.** A solution of the ethyl methanetrisulfonate in hydrochloric acid was prepared and the reaction was carried out at 25.0° C in a glass-stoppered flask from which samples were removed from time to time with a five or ten ml pipette. After the addition of a few drops of 0.01 per cent solution of methyl orange the samples were immediately titrated with a bromine solution until the indicator color just disappeared. The small consumption of the indicator of the bromine solution was determined, and the correction was substracted from the values obtained. From these results the actual ester concentration $c_x$ was calculated. A solution of 0.5 *M* potassium bromide and about 0.003 *M* bromine was used for these titrations. Control experiments verified that the ethyl alcohol formed had no effect on the results. Fig. 1 shows a curve from a hydrolysis experiment in 0.100 *N* HCl with $c_0 = 0.01000$ *M*. If $k = \frac{2.303}{t} \log c_0/c_x$, the slope of the line gives a value of $k$ of $3.36 \cdot 10^{-2}$. Another experiment with 0.050 *N* HCl and $c_0 = 0.01000$ *M* gives $k = 1.89 \cdot 10^{-2}$.

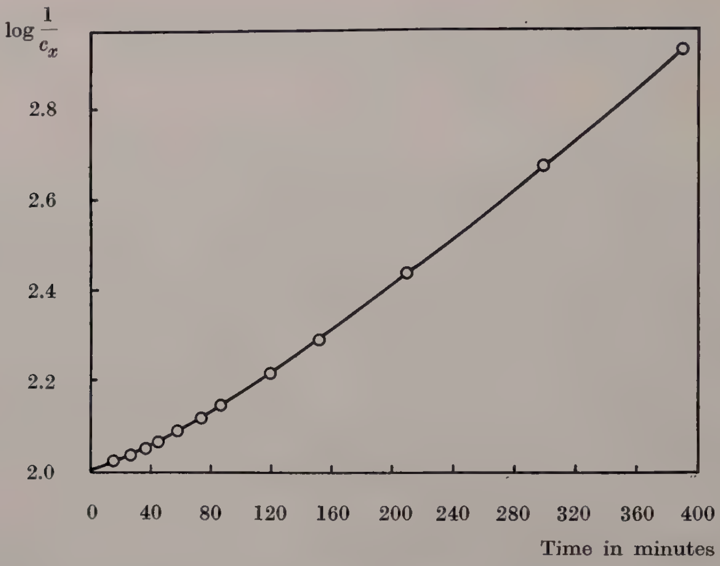


Fig. 2. Hydrolysis of ethyl methanetrisulfonate in water. $c_0 = 0.01$ *M.*

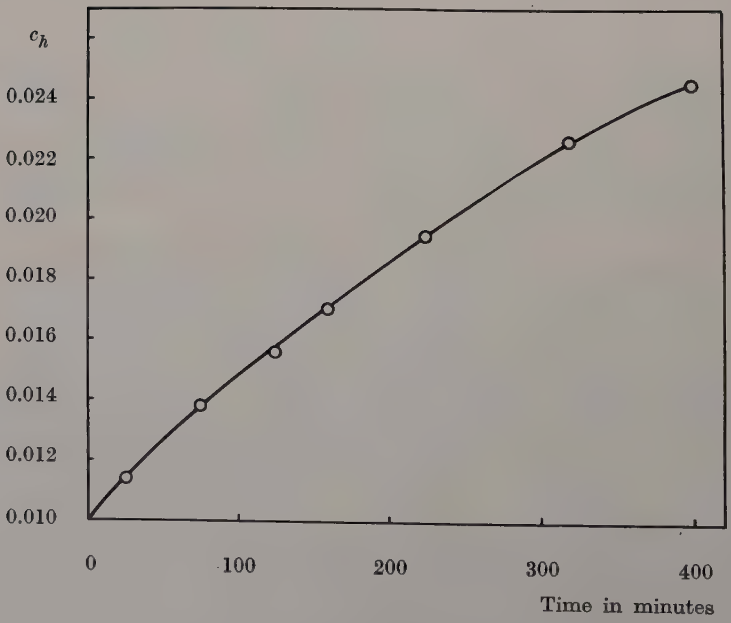


Fig. 3. Titration with alkali. $c_0 = 0.01$ *M.*

*Table 1*

Hydrolysis of ethyl methanetrisulfonate in water. $c_0 = 0.01000\ M$.

| $t$ | $V$ | $c_x$ | $\frac{1}{t}\log c_0/c_x$ | $\int_0^t c_h\,dt$ | $k_2$ |
|---|---|---|---|---|---|
| 17 | 15.20 | 0.00945 | $1.43 \cdot 10^{-3}$ | 0.179 | 0.314 |
| 27 | 14.69 | 914 | 1.45 | 0.290 | 0.311 |
| 37 | 14.19 | 883 | 1.47 | 0.408 | 0.306 |
| 45 | 13.82 | 860 | 1.46 | 0.505 | 0.300 |
| 58 | 12.91 | 803 | 1.64 | 0.670 | 0.327 |
| 74 | 12.09 | 752 | 1.67 | 0.884 | 0.323 |
| 87 | 11.37 | 707 | 1.73 | 1.065 | 0.325 |
| 120 | 9.71 | 604 | 1.83 | 1.555 | 0.324 |
| 153 | 8.19 | 509 | 1.91 | 2.088 | 0.323 |
| 210 | 5.87 | 365 | 2.08 | 3.106 | 0.324 |
| 300 | 3.39 | 211 | 2.25 | 4.944 | 0.315 |
| 390 | 1.90 | 118 | 2.38 | 7.028 | 0.304 |
| | | | | Mean value 0.316 | |

The hydrolysis experiment in aqueous solution in the absence of a mineral acid does not result in a straight line when $\log c_x$ is plotted against time (Fig. 2). This is due to the variation of the hydronium ion concentration. The hydrolysis is therefore followed by titration with alkali and a curve is constructed by plotting $c_h$ against time $t$ (Fig. 3). From this diagram the area $\int_0^t c_h\,dt$ is evaluated and the bimolecular constant $k_2$ obtained, using equ. (1). The results of such an experiment are shown in table 1. The second column indicates the required $V$ ml of the bromine solution of concentration 0.00311 $M$. The fourth column shows the unimolecular "constant" (log). In column five the areas from Fig. 3 are tabulated, and the sixth column shows the values of $k_2$. From this it seems that the agreement is comparatively good. If the constant $k$ from Fig. 1 is divided by $c_h$ 0.11 and 0.06 respectively, the bimolecular constant $k_2$ is found to be 0.305 and 0.315, of which the latter is in good agreement with the value of $k_2$ from table 1.

Experiment with a solution of the ester in sodium hydroxide shows that the decrease of the concentration of alkali is slow. The anion of the trisulfonic acid appears to be unstable in alkali solution. It was found that a solution of potassium methanetrisulfonate in about 0.03 $N$ NaOH used up alkali. The solution after acidifying reduced permanganate. Probably sulfite had been produced.

## SUMMARY

Ethyl methanetrisulfonate has been prepared. M. P. 75° C.
The ester reacts rapidly with bromine in several solvents.
The first stage reaction in the hydrolysis of the ester has been studied, and

this reaction was found to be catalyzed by hydronium ions. In aqueous solution the bimolecular constant has the value of 0.316 liters $\text{moles}^{-1}$ $\text{min}^{-1}$. In 0.050 $N$ and 0.100 $N$ hydrochloric acid the pseudounimolecular constants are found to be $1.89 \cdot 10^{-2}$ and $3.36 \cdot 10^{-2}$ $\text{min}^{-1}$.

Laboratory of Organic Chemistry, University of Uppsala, February 1951.

REFERENCES. 1. **E. Samén**, Arkiv Kemi *1*, 231 (1949). — 2. ——, Acta Chem. Scand. *4*, 397 (1950).

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# Zur Methodik der Protease-Bestimmung in Tierorganen.

Von **Hans von Euler** und **Hans Hasselquist**

Die Schwierigkeiten, die den enzymatischen Wirkungen zugrundeliegenden Enzymmengen zu bestimmen, machen sich besonders bezüglich der proteolytischen Enzyme der Tierorgane geltend, welche bekanntlich zum grossen Teil als ,,Desmo-Enzyme“ an die Zellen fest gebunden sind. Trotz der eingehenden Versuche von Willstätter und seiner Mitarbeiter ist die quantitative Erfassung dieser ,,Desmo-Enzyme“ bis jetzt nur sehr unvollständig gelungen. ,,In der Tat,“ schreiben Willstätter und Rohdewald in einer ihrer letzten Arbeiten[1], ,,windet sich der Weg oft durch ein Labyrinth von Klippen, von Unstimmigkeiten und Widersprüchen. Durch ihre Berücksichtigung drohen die Abhandlungen schwerfällig und unübersichtlich zu werden. Aber man darf den Unstimmigkeiten nicht ausweichen, die Abweichungen nicht übersehen oder ausglätten. Der scheinbare Fehler von heute wird morgen Ausgangspunkt einer neuen Arbeit. Der Widerspruch, den man zu verstehen lernt, ist eine Entdeckung.”

Die hier vorliegende Aufgabe, eine geeignete Methode zur Bestimmung der Proteasen in Organen von Ratten und Meerschweinchen zu finden, war veranlasst durch die Studien dieses Arbeitskreises über *experimentellen Rheumatismus*[2]. In der ersten, an Ratten ausgeführten, und in einer zweiten an Meerschweinchen begonnenen Untersuchung wurde zunächst angestrebt, an diesen Tieren arthritische Symptome hervorzurufen, und zwar durch Behandlung derselben mit grossen Dosen von Desoxycorticosteron (Doca) nach den Angaben von Selye[3] in Kombination mit Ascorbinsäure-armer Diät nach Schaffenburg, Masson und Corcoran[4] und mit Streptococcen-Injektion.

An den so behandelten Meerschweinchen soll der Zusammenhang zwischen arthritischen Symptomen und den an einigen ihrer Organe auftretenden enzymatischen Veränderungen festgestellt werden, und zwar wurde dazu zunächst der *Kathepsin-Gehalt* (die Kathepsin-Wirkung) der Milz, der Leber und der Nieren studiert.

Nach einer Reihe von älteren, bis 1901 zurückgehenden Versuchen über Organproteasen haben Waldschmidt-Leitz und Deutsch[5] 1927 gefunden, dass man in der Milz „nur zwei proteolytische Enzyme zu unterscheiden hat, eine Protease vom Reaktionsoptimum pH = 4, spezifisch für die Hydrolyse von Proteinen und ohne Wirkung auf einfache Peptide, und daneben in überwiegender Menge ein peptidspaltendes Enzym, dessen optimale Reaktion von pH = 8,0 der anderer bekannter Peptidasen entspricht."

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[1] R. Willstätter u. M. Rohdewald, Z. physiol. Chem. 229, 241 (1934). — Siehe auch ebenda 208, 258 (1932).

[2] Euler u. L. Heller, Dieses Arkiv 3, Nr 13 (1951).

[3] Hans Selye, O. Sylvester, C. E. Hall u. C. R. Leblond, J. Am. Med. Ass. 124, 201 (1944)

[4] C. Schaffenburg, G. M. C. Masson u. c. Corcoran, Proc. Soc. exp. Biol. a. Med 74, 358 (1950)

[5] E. Waldschmidt-Leitz u. W. Deutsch, Z. physiol. Chem. 167, 285 (1927)

Bald darauf bestätigten und erweiterten Willstätter und Bamann[1] diesen Befund; sie schrieben: „So wie E. Waldschmidt-Leitz und W. Deutsch an den Lienproteinasen gezeigt haben, so scheint es allgemeiner zuzutreffen, dass nur eine im schwach sauren Gebiet wirkende Proteinase und eine bei schwach alkalischer Reaktion wirkende Peptidase für die beobachteten Spaltungen verantwortlich sind, und dass auf ihr Zusammenwirken auch scheinbare tryptische Effekte zurückzuführen sind." ... „Es lässt sich kaum umgehen, für die bei schwach saurer Reaktion wirkende Proteinase ... einen besonderen Namen einzuführen; wir schlagen dafür die Bezeichnung *Kathepsin* vor (von καθέψειν = verdauen)". ... „Es bedarf also weiterer Untersuchungen, um zu entscheiden, ob nicht Pepsin und Trypsin allein in wahren Lösungen sezerniert werden, Kathepsin und Erepsin hingegeben in Form von Granula und ihren Suspensionen auftreten." Wesentliche Erweiterungen unsrer Kenntnisse der Kathepsine, besonders der Nieren-Kathepsine verdankt man Max Bergmann[2], der 4 verschiedene Kathepsine unterscheidet, auf die wir später noch zurückkommen.

Willstätter und Bamann hatten ihr Kathepsin zunächst aus Magenschleimhaut von Schweinen dargestellt. In diesem Material liegt nach Willstätter und Rohdewald[3] das Kathepsin teils als Lyo-, teils als Desmo-Enzym vor, und kann durch wasserfreies Glycerin in diese beiden Formen getrennt werden.

Waldschmidt-Leitz und Deutsch verrieben den aus der Fleischmaschine kommenden Milzbrei mit dem doppelten Volumen 87 %igen Glycerins und liessen diese Suspension nach Zusatz von Essigsäure 4–15 Stunden stehen bevor sie filtriert wurde.[4]

L. Anson[5], der Rinderleber als Ausgangsmaterial für Kathepsin verwendete, zerkleinerte das gefrorene Organ in der Fleischmaschine und überliess den Brei mit der doppelten Gewichtsmenge Wasser und mit 15 % Toluol über Nacht bei Zimmertemperatur der Hydrolyse. Die filtrierte Lösung wurde dann mit HCl angesäuert und mit Ammoniumsulfat von Hämoglobin befreit.

Als Substrat ist in den erwähnten Untersuchungen von Willstätter, Anson u. a. Casein, Edestin oder Hämoglobin verwendet worden. Für die Bestimmung des hydrolysierten Betrages der Proteine sind verschiedene Methoden zur Anwendung gekommen, unter denen die Titration in alkoholischer Lösung (Willstätter), die nephelometrische Methode von Rona u. Kleinmann und die colorimetrische Bestimmung des abgespaltenen Tyrosins nach Anson[6] zu nennen sind.

Bei den Versuchen, für welche diese methodische Arbeit unternommen wurde, handelt es sich weniger um die Feststellung qualitativer, als *quantitativer* Veränderungen des Proteingehaltes, welche in den Organen der Versuchstiere nach ihrer verschiedenen Vorbehandlung eintreten. Erforderlich war also in erster Linie eine möglichst vollständige Erfassung (Extraktion) der Proteasen jedes Organs, unter denen ja das Kathepsin die Hauptmenge ausmacht.

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[1] R. Willstätter u. E. Bamann, Z. physiol. Chem. 180, 127 (1928)

[2] M. Bergmann, J. Fruton u. G. W. Irving, J. Biol. Chem. 138, 249 (1941)

[3] R. Willstätter u. M. Rohdevald, Z. physiol. Chem. 208, 258 (1932)

[4] Kathepsin-Darstellungen aus den alkohol-behandelten, getrockneten Organen haben Karrer u. Zehender, ferner Maschmann u. Helmert sowie Purr angegeben.

[5] L. Anson, J. gen. Physiol. 20, 565 (1937). —

[6] L. Anson, J. gen. Physiol. 22, 79 (1939).

Das unter Zusatz von 8 ml Borat-HCl-Pufferlösung (pH = 3,57) zu je 1 g Organ zerriebene Material wurde deswegen durch Homogenisieren zwischen schnell rotierenden Glasschliffen fein verteilt. Da durch das Homogenisieren das Kathepsin noch keineswegs ganz von den Zellen freigemacht bezw. in Lösung gebracht wird, haben wir versucht, ob durch 1-tägiges Stehen des homogenisierten Organbreies bei 0° mehr Kathepsin in Lösung geht. Versezt man den homogenisierten Organbrei teils sofort, teils nach 24 Stunden mit der Hämoglobinlösung und dann mit Trichloressigsäurelösung und filtriert die dadurch enstandene Fällung durch Filtrierpapier, so zeigen die mit den Filtraten ausgeführten Bestimmungen der $NH_2$-Abspaltung (Kjeldahl-Bestimmungen), dass nach dem Stehen des Homogenates die Aktivität des Filtrates nicht immer zunimmt sondern zuweilen nach 1-tägigem Stehen (Leber und Niere von Meerschweinchen) sehr stark sinkt (nach 2-tägigem Stehen trat diese Aktivitätsabnahme in manchen Fällen noch stärker hervor).

In Übereinstimmung damit ergab sich, dass die klaren Enzym-haltigen Filtrate bei längerem Stehen bei +5° einen Niederschlag absetzen, der offenbar einen wesentlichen Teil des extrahierten Kathepsins enthielt, denn nach dem Abscheiden des Niederschlags erwies sich die Lösung inaktiv.

Die Organ-Extrakte enthalten ausser dem Kathepsin auch andere N-liefernde Stoffe. So erhält man z. B. mit einem in oben beschriebener Weise hergestellten frischen Nieren-Extrakt (Meerschweinchen) 0,057 mg N per 2 ml Extrakt.

1 ml Nieren-Extrakt (Meerschweinchen) ergaben bei zwei Mikro-Kjeldahl-Bestimmungen

$$N = 2{,}32 \text{ und } 2{,}24$$

2 ml Reaktionslösung bei der Kathepsin-Bestimmung enthalten

| | Anfangswerte mg N | Filtrat der Trichloressigsäurefällung (ohne enzym. Hämoglobinspaltung) mg N (nicht fällbares N) |
|---|---|---|
| Nieren-Extr. (Enzym) | 0,28; 0,29 | 0,057; 0,057 |
| Hämoglobin | 1,205; 1,215 | 0,038; 0,039 |

Als Protein-Substrat zur Ermittlung der Kathepsin-Aktivität verwendten wir käufliches Hämoglobin. 2,5 g Hämoglobin, dem wir Merthiolat im Verhältnis 1: 40000 zusetzten, wurden in 100 ml Wasser möglichst vollständig gelöst. Die centrifugierte, klare Lösung enthielt 38 mg Hämoglobin in 2 ml, war also 1,9%ig. Von dieser Hämoglobinlösung entsprachen 2 ml dem Wert 2,42 mg N (vgl. obige Tabelle).

Um die Reproduzierbarkeit der Trichloressigsäure-Fällung zu prüfen wurden in Doppelbestimmungen je 4 ml der 1,9 %igen Hämoglobinlösung mit 2 ml Borat/HCl-Pufferlösung versetzt. Dazu wurden 10 ml 0,3 n. Trichloressigsäurelösung gegeben, worauf vom Niederschlag abfiltriert wurde.

| | | |
|---|---|---|
| Probe I: | Trichloress.-Zugabe sofort | 0,039 mg N |
| Probe II: | » » nach 60 Min. 37° | 0,038 mg N |

Zur Mikro-Kjeldahl-Analyse wurden je 2 ml des Filtrates verwendet.

Zu jeder Analyse wurden folgende Mengen gemischt:

4 ml Hämoglobinlösung wurden mit 1 ml Borat-Pufferlösung 10 Minuten bei 37° inkubiert und gleichzeitig wurde der Kathepsin-haltige Organ-Extrakt (frisch oder nach 24-stündigem Stehen) bei 37° mit der Borat-Pufferlösung gemischt. Nach 10 Minuten wurden 5 ml Hämoglobinpufferlösung + 1 ml der Kathepsin-haltigen Organextrakt-Pufferlösung bei 37° mit 10 ml Trichloressigsäurelösung versetzt. Die dadurch entstandene Eiweissfällung wurde abfiltriert. Von diesem Filtrat wurden 2 ml zur Mikro-Kjeldahl-Bestimmung verwendet.

In unserem ursprünglichen Reaktionsgemisch kommt also auf 0,285 Organextrakt-N eine Hämoglobin-N-Menge von 1,21 mg. Es stammen also etwa 80 % des gesamten Stickstoffs vom Hämoglobin und nur 20 % vom Organextrakt. Die N-haltigen Komponenten der Organextrakte werden aber meist langsamer gespalten als das Hämoglobin bezw. dessen Globin und kommen also bei unserer Versuchsanordnung kaum in Betracht.

Innerhalb unserer Reaktionszeit, 60 Minuten werden im Versuch mit Meerschweinchen-Niere bei 37° rund 3 % des aus dem Hämoglobin abspaltbaren N freigemacht.[1]

Der oben erwähnten Untersuchung über den Einfluss des Desoxycorticosterons auf den Kathepsin-Gehalt der Niere und Milz von Meerschweinchen entnehmen wir folgende Zahlen, geltend für eine Reaktionsdauer von 60 Minuten bei 37°:

| Organ | mg N abgespalten in 1 ml Organextrakt (Mittelwert) | |
|---|---|---|
| | Alter des Extraktes | |
| | Frisch | 24 Stunden |
| Niere | 0,074 | 0,074 |
| Milz | 0,038 | 0,100 |

Bei weiterer Ausarbeitung der Methodik der Kathepsin-Bestimmung wird man auch des Homogenat nicht bei 0° sondern bei einer höheren Temperatur, etwa 37° extrahieren.

Auch ist eine Extraktion der mit Alkohol-Aceton-Äther entwässerten Organe in Betracht zu ziehen, wobei ausser dem Lyo-Enzym auch wenigstens ein Teil des Desmo-Enzyms freigelegt wird. Eine Unsicherheit wird bei der von uns (wie auch von früheren Forschern) eingehaltenen Arbeitsweise durch die Homogenisierung der frischen oder vorher gefrorenen Organe veranlasst. Besonders wenn es auf quantitative Vergleiche der Kathepsin-Wirkung eines Organes bei variierender Vorbehandlung ankommt, erweist sich die Homogenisierung nicht hinreichend gleichheitlich, und eine teilweise Zerstörung der Enzyme ist anscheinend schwer zu vermeiden.

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[1] In reaktionskinetischer Hinsicht wäre es vorzuziehen gewesen, statt 60 Minuten eine längere Reaktionszeit zu wählen, bei welchen etwa die Hälfte des aus dem Hämoglobin abspaltbaren Stickstoffs als Reaktionsprodukt erhalten wird.

Die hier vorliegende Beschreibung unseres Verfahrens der Kathepsin-Bestimmung berührt, wie schon erwähnt, nicht das Ausgangsproblem: „Proteinstoffwechsel bei arthritischen Schäden", über dessen Bearbeitung EULER und HELLER an anderer Stelle berichten werden.

Dagegen soll in einer Fortsetzung dieser methodischen Arbeit untersucht werden, inwieweit Aktivatoren und Hemmungskörper an der Enzymwirkung unserer Organextrakte beteiligt sind. Dass Glutathion und Cystein manche proteolytischen Reaktionen beeinflussen geht schon aus Arbeiten von MAX BERGMANN[1] hervor. Dieser Forscher unterschied 4 Kathepsine (intracellulare Proteinasen), welche verschiedene Substrate spezifisch angreifen; von diesen erfordern Kathepsin II, III und IV Cystein zur Spaltung synthetischer Peptide.[2]

Besonders bemerkenswert ist, dass einfache Peptide durch Cystein-aktivierte Proteinasen *synthetisiert* werden können. Für die Beurteilung des Proteinstoffwechsels arthritischer Organe spielen zweifellos die synthetischen Protease-Wirkungen eine bedeutende Rolle.

Herrn Ingenieur ROGNE ANDER danken wir für seine Mitwirkung bei der Bestimmung der Hämoglobin-Spaltungen.

Stockholm, Vitamin-Institut und Institut für organ.-chemische Forschung der Universität.

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[1] MAX BERGMANN u. Mitarb. J. Biol. Chem. 124, 1 u. 7 (1938)

[2] J. S. FRUTON, G. W. IRVING u. M. BERGMANN, J. Biol. Chem. 141, 763 (1941).

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# Acetic acid metabolism in *Rhodospirillum rubrum* under anaerobic condition. II.

By C. Cutinelli, G. Ehrensvärd, L. Reio, E. Saluste and R. Stjernholm

At present, very little is known about the formation of amino acids in microrganisms under anaerobic condition, as compared with aerobic systems, where especially isotope techniques have contributed to the elucidation of some steps in the schemes of general metabolism. The use of double-labelled acetate, $C^{13}H_3—C^{14}OONa$, as the sole source of carbon for the growth of *Torulopsis utilis* and *Escherichia coli* has given some information about the metabolic connection between acetate and amino acids (1, 2, 3, 4, 5) based on the localization and determination of the isotope content in different carbon atoms of amino acids, originating from the $C^{13}H_3$- or the $C^{14}OOH$-group of acetic acid. As an analogue to these experiments with aerobic organisms we have cultivated *Rhodospirillum rubrum* under *anaerobic* condition, in the presence of isotopic acetate. The results of some preliminary experiments of this work is given in part I of this series (6).

*Rhodospirillum rubrum,* as shown by van Niel (7) grows in light anaerobically, on acetate in the presence of a certain amount of bicarbonate (plus a small amount of yeast extract, providing growth factors). In our preliminary experiments we cultivated Rhodospirillum in two series on the same, but differentially labelled substrate in order to be able to account for the contribution of the methyl-and the carboxyl group of acetate and the bicarbonate carbon to the formation of bacterial protein. The series were:

I. $C^{13}H_3C^{14}OONa + NaHCO_3$
II. $CH_3COONa + NaHC^{14}O_3$

The result was that about 40 % of the total carbon of trichloro-acetic acid precipitable protein originated from the methyl group of acetate, 22 % from the carboxyl and 12 % from the bicarbonate. This cultivation was done in small scale under 96 hours. Since the over-all incorporation of isotope in the protein was high we tried a similar cultivation in large scale during a ten hour period, whereby an over-all isotope labelling was obtained, sufficient for a subsequent determination in isolated carbon fragments of different amino acids. This paper gives the result of the localization of the isotope content in different atoms of alanine, aspartic acid and glutamic acid, isolated from hydrolysates of Rhospirillum from both cultivation series.

The $C^{13}H_3C^{14}OONa$ and $NaHC^{14}O_3$ of the two series of media were prepared according to standard procedures as described by *Baddiley, Ehrensvärd* et al. (1) and *Calvin* et al (8). The amounts prepared and the isotope content were as follows:

| | | |
|---|---|---|
| $C^{13}H_3C^{14}OONa$, water-free, 72 g. | $C^{13}$: | 3.55 atom per cent excess |
| | $C^{14}$: | 26 300 counts per minute per 15 mg of $BaCO_3$. |
| $NaHC^{14}O_3$, 48 g. | $C^{14}$: | 46 800 counts per minute per 15 mg of $BaCO_3$ |

The bicarbonate, as made up from a small sample (100 mg) of high activity by dilution with 47.9 g non-labelled sodium bicarbonate, was further diluted in the medium by the presence of 48 g of non-labelled potassium bicarbonate, so that the actual $C^{14}$-activity of the bicarbonate carbon in the medium was 26 100 counts per minute. (In all isotope determinations the carbon material was converted to barium carbonate samples; the activity of $C^{14}$ being measured under standard condition on 15 mg of $BaCO_3$ on 1 sq. cm discs. $C^{13}$-determinations were carried out on the same samples, after the $C^{14}$-determination, with aid of mass-spectrometric analysis.)

The media were as follows:

| *Series I.* | | *Series II.* | |
|---|---|---|---|
| $C^{13}H_3C^{14}OONa$, water-free: | 72.0 g | $CH_3COONa$, water-free: | 72.0 g |
| $NaHCO_3$ | 48.0 g | $NaHC^{14}O_3$ | 48.0 g |
| $KHCO_3$ | 48.0 g | $KHC^{14}O_3$ | 48.0 g |
| $NH_4Cl$ | 24.0 g | $NH_4Cl$ | 24.0 g |
| $MgSO_4 + 7\,H_2O$ | 4.8 g | $MgSO_4 + 7\,H_2O$ | 4.8 g |
| $CaCl_2 + 6\,H_2O$ | 0.9 g | $CaCl_2 + 6\,H_2O$ | 0.9 g |
| Yeast extract (Difco) | 12.0 g | Yeast extract (Difco) | 12.0 g |
| HCl, 6N | 108.0 ml | HCl, 6N | 108.0 ml |

in 24 litres of sterile tap water.

### *Cultivation procedures.*

1 000 ml of sterile, *non-isotopic*, medium of the composition given above in a one-litre bottle was inoculated with a small amount of a pure culture of *Rhodospirillum rubrum*, grown on Difco-Agar under anaerobic condition. (100 ml of sterile tap water, 2.5 g of Agar, 0.3 g of Difco yeast extract; pH adjusted to 7.0 by bicarbonate.) Before closing the bottle, pure nitrogen was passed through the suspension for 15 minutes. The tightly stoppered bottle was then kept at 28° under continuous illumination with three 100-watt electric bulbs. After four days the bottle was opened and the content directly transferred to 5 litres of the same sterile medium in a 6 litre Pyrex Erlenmeyer flask. After flushing out with nitrogen, the flask was stoppered and illuminated as before at 28° for four days. Then the bacteria were centrifuged off under sterile condition and resuspended in 1 litre of the same medium.

Eight 6 litre flasks, each containing 5 875 ml of medium, still non-isotopic, were then inoculated with each 125 ml of the bacteria-suspension, flushed with nitrogen, stoppered and illuminated in a light-cabinet, containing nine 100 watt

*Table I.*

Isotope content of different carbon atoms of aspartic acid isolated from *Rhodospirillum rubrum*, cultivated on acetate and bicarbonate, labelled according to series I and II. $C^{13}$ is given in atom per cent excess, $C^{14}$ is given in counts per minute per 15 mg of barium carbonate.

| | Series I | | Series II |
|---|---|---|---|
| | $C^{13}$ | $C^{14}$ | $C^{14}$ |
| *Substrate:* | | | |
| Series I, $C^{13}H_3C^{14}OONa$ | 3.55 | 26 300 | — |
| $NaHCO_3$ | — | — | — |
| Series II, $CH_3COONa$ | — | — | — |
| $NaHC^{14}O_3$ | — | — | 26 100 |
| Aspartic acid, total combustion | 0.205 | 830 | 1 225 |
| » , carboxyls, α : β = L : 1 (by ninhydrin) | 0.055 | 750 | 2 735 |
| » , carboxyls, α : β = 4 : 1 (by Chloramine T) | 0.075 | 798 | 2 220 |
| » , carboxyls, α : β = 6, 3 : 1 (by Chloramine T) | 0.07 | 742 | 2 460 |
| » , α- and β-carbon atoms calculated mean value | 0.32 | 910 | < 40 |
| » , β-carbon atom, as iodoform | 0.50 | 650 | < 100 |
| *Compiled values:* | | | |
| α-carboxyl | 0.08 ± 0.02 | 780 ± 50 | 2 300 ± 220 |
| α-carbon atom | 0.14 + 0.06 | 1 170 ± 230 | 0 |
| β-carbon atom | 0.50 ± 0.05 | 650 ± 70 | < 100 |
| β-carboxyl | 0.03 ± 0.01 | 730 ± 120 | 3 000 ± 200 |
| *In medium at the end of experiment:* | | | |
| Bicarbonate carbon | 0.075 | 1 025 | 26 000 |
| Acetate carboxyl carbon | 0.02 | 26 000 | 220 |
| Acetate methyl carbon | 3.55 | 150 | 20 |

bulbs. During the following five days dry weight estimations, carried out on small samples of the cultures, showed that for each flask the original inoculum, about 0.5 g, had increased to between 4.0–4.5 g (dry weight) at the end of the five-day period.

The bacteria of each of the eight flasks were then used as the inoculates of four similar flasks, containing isotopic acetate (series I) and four flasks containing isotopic bicarbonate (series II). One by one, the eight flasks were opened, the content centrifuged under sterile condition, and the bacterial material transferred alternately to the flasks of series I and II, each containing 6 litres of isotopic medium. The time interval between each transfer was kept as low as possible, about 2 hours. The four flask of series I and the four of series II were then flushed with nitrogen, stoppered and illuminated for 10 hours. At the end of the experiment the bacterial material from each series was separated off by centrifugation in a Sharples centrifuge, resuspended in water and washed by centrifugation several times in order to wash out the isotopic media. Then the material was treated three times with absolute alcohol

*Table II.*

Isotope content of different carbon atoms of alanine and glutamic acid, isolated from *Rhodospirillum rubrum*, cultivated on acetate and bicarbonate, labelled according to series I and II. $C^{13}$ is given in atom per cent excess, $C^{14}$ in counts per minute per 15 mg of barium carbonate.

| | Series I | | Series II |
|---|---|---|---|
| | $C^{13}$ | $C^{14}$ | $C^{14}$ |
| *Substrate:* | | | |
| Series I, $C^{13}H_3C^{14}OONa$ | 3.55 | 26 300 | — |
| $NaHCO_3$ | — | — | — |
| Series II, $CH_3COONa$ | — | — | — |
| $NaHC^{14}O_3$ | — | — | 26 100 |
| Alanine, carboxyl (by ninhydrin) | 0.025 | 210 | 2 350 |
| » , α- and β-carbon atoms, by comb. of acetaldehyde-thio-semicarbazone | 0.28 | 1 700 | 30 |
| Alanine, β-carbon atom as iodoform | 0.56 | 100 | 15 |
| *Compiled values:* | | | |
| Carboxyl | 0.03 ± .01 | 210 ± 20 | 2 350 ± 50 |
| α-carbon atom | 0.06 ± .03 | 3 300 ± 150 | < 40 |
| β-carbon atom | 0.50 ± .05 | 100 ± 10 | 15 |
| Glutamic acid, total combustion | 0.27 | 1 590 | 815 |
| » , α-carboxyl (by ninhydrin) | 0.055 | 580 | 3 035 |
| » , γ-carboxyl (by Schmidt reaction) | 0.000 | 4 900 | 190 |
| *Compiled values:* | | | |
| α-carboxyl | 0.055 ± .005 | 580 ± 30 | 3 035 ± 180 |
| γ-carboxyl | 0.000 | 5 000 ± 500 | 200 ± 40 |
| α-,β-,γ-carbon atoms, calculated mean value | 0.43 ± .03 | 820 ± 120 | < 280 |

and five times with ether, by centrifugation. The final products were kept at 60° for some time. Yield 18.0 g of series I and 14.7 g of series II.

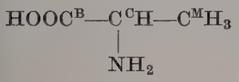
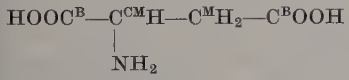
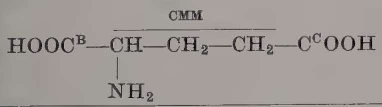
The alcoholic and the ether extracts contained considerable amounts of green and red pigments. (6).

## *The isotope distribution in alanine, aspartic acid and glutamic acid.*

The dry bacterial material from series I and II was subjected to hydrolysis by treatment with 20 % hydrochloric acid at 100° for 24 hours. After separating off some humine the acid hydrolysates were evaporated to dryness and the amino acids from both hydrolysates separately isolated according to procedures described in previous papers (4.5). Alanine, aspartic acid and glutamic acid were subjected to special degradation procedures (4.5) in order to localize the isotope content in different carbon atoms, originating from the methyl group and the carboxyl of acetate (in series I) and the bicarbonate carbon (in series II).

*Table III.*

Survey of isotope distribution in alanine, aspartic acid and glutamic acid. The isotope values, taken from Tables I and II, are given in per cent of the corresponding isotope content in the methyl group and carboxyl of acetate, and the bicarbonate present in the medium at the beginning of the experiment. The main origin of the different carbon atoms of the amino acids is given, using the index symbols: M for the methyl group, C for carboxyl and B for bicarbonate, as the source of the amino acid carbon atoms.

| | $C^{13}$(M) | $C^{14}$(C) | $C^{14}$(B) | All sources |
|---|---|---|---|---|
| *Substrate:* | | | | |
| Acetic acid: carboxyl | — | 100 | — | |
| methyl group | 100 | — | — | |
| Bicarbonate: | — | — | 100 | |
| Alanine: carboxyl | 0.9 | 0.8 | 9.1 | 10.8 |
| α-carbon atom | 2.0 | 12.6 | 0.0 | 14.5 |
| β-carbon atom | 14.0 | 2.0 | 0.0 | 16.0 |
|  $\mathrm{HOOC^{B}{-}\underset{NH_2}{C^{C}H}{-}C^{M}H_3}$ | | | | Mean 13.8 |
| Aspartic acid: α-carboxyl | 2.3 | 2.9 | 8.7 | 13.9 |
| α-carbon atom | 4.0 | 4.9 | 0.0 | 8.9 |
| β-carbon atom | 14.0 | 2.2 | 0.0 | 16.2 |
| β-carboxyl | 1.0 | 2.8 | 11.4 | 14.6 |
|  $\mathrm{HOOC^{B}{-}\underset{NH_2}{C^{CM}H}{-}C^{M}H_2{-}C^{B}OOH}$ | | | | Mean 13.4 |
| Glutamic acid: α-carboxyl | 1.9 | 2.2 | 11.5 | 15.6 |
| γ-carboxyl | 0.0 | 18.3 | 0.5 | 18.3 |
| α-,β-,γ-carbon atoms, mean value | 10.0 | 3.1 | 0.6 | 13.7 |
|  $\mathrm{HOOC^{B}{-}\overset{CMM}{\overline{\underset{NH_2}{CH}{-}CH_2{-}CH_2}}{-}C^{C}OOH}$ | | | | Mean 15.0 |

The result of these degradation procedures is shown in Tables I and II. In Table III a survey of the isotope distribution in the the three amino acids is given, based on the primary data of Tables I and II.

## *Discussion.*

The result of the degradations shows that the labelled bicarbonate carbon of series II does *not* enter into all positions of the carbon structures of the three amino acids, which is contrary to what would be expected in an organism containing a photosynthetically active system. The acetate carboxyl, on the other hand, seems to contribute more or less to almost all carbon atoms of the amino acids in a manner, which indicates at least two special reaction types.

1. The enormous $C^{14}$-content from the acetate carboxyl in the $\gamma$-carboxyl of glutamic acid, as compared with that of the $\alpha$-carboxyl, indicates that the $\gamma$-part of the glutamic acid and thus that of the $\alpha$-keto-glutaric acid is derived directly from acetate. Assuming a condensation of acetate and oxal-acetate to ketoglutarate, via citrate, the $\alpha$-carboxyl of the former should consequently emanate from one of the oxal-acetate carboxyls. The values of the aspartic acid carboxyls clearly show that their main origin is from the $CO_2$-carbon and not the acetate carboxyl, which supports the idea of an acetate-oxal-acetate condensation.

2. The clear-cut isotope distribution in alanine; the $\beta$-atom from the methyl group of acetate, the $\alpha$-atom from the carboxyl of acetate and the alanine carboxyl from the bicarbonate pool, point to a function of the acetate which is in concordance with the view, expressed by *Calvin* (9, 10). The mechanism, proposed by Calvin, of a 2-carbon compound adding $CO_2$, forming phosphoglyceric acid, must as a consequence result in the formation of a pyruvic acid, and thus of alanine, the carboxyl of which is primarily derived from $CO_2$, the remainder from the 2-carbon compound. It looks from our experiments as if this 2-carbon compound could be closely related to acetate, probably a reduction product. In that case the reduction of acetate to a reactive 2-carbon compound should be the main function of the photosynthetic mechanism in *Rhodospirillum* in light, under anaerobic condition.

The labelling of alanine, reflecting the labelling of pyruvic acid $C^{13}H_3–C^{14}O–C^{14}OOH$, should to some extent occur in the labelling of oxal-acetate, as shown by aspartic acid. If a secondary fixation of $CO_2$ to pyruvic acid takes place (analogous to the Wood-Werkman-reaction in aerobic systems (11, 12)) one would expect in aspartic acid a labelling of the "alanine"-type, plus a $\beta$-carboxyl, originating from $CO_2$. This is in fact the case, and the non-identical labellings of the $\alpha$- and the $\beta$-carbon atoms point, in addition, to the absence of a close equilibrum with a symmetrical metabolite, like fumarate or succinate. This is a feature, which is in direct contrast with the findings in aerobic systems, such as *Torulopsis utilis* and *Escherichia coli*, grown on labelled acetate as the sole source of carbon (4, 5).

The absence of any $C^{13}$ in the $\gamma$-carboxyl of glutamic acid of series I indicates that the acetate is not to any greater extent involved in reactions, in which its methyl group could contribute to the acetate carboxyl, as it has been shown in *Rhodospirillum* under aerobic condition in the dark (13). This is furthermore supported by direct $C^{13}$-determination in the carboxyl of the acetate of series I, recovered after the end of the anaerobic cultivation. It was found that this $C^{13}$-content was only 0.02 per cent excess. In addition, the $C^{14}$-content of the acetate methyl group was 150 counts per minute. These values represent 0.56 and 0.57 per cent of the isotope content, originally present in the acetate of the medium I. It is obvious that the $C^{13}$-content of the acetate carboxyl formed during the experiment is too small to be detected in the $\gamma$-carboxyl of glutamic acid of series I.

The $C^{13}$- and $C^{14}$-content of the carbonate carbon, recovered from series I, after the end of the experiment, 0.075 per cent excess $C^{13}$ and 1 025 counts per minute $C^{14}$, point to a different rate of decarboxylation reactions, with respect to the methyl and the carboxyl of acetate. The ratio was 1:1, 85, which means that about twice as much carboxyl carbon has been taken off as $CO_2$ than methyl carbon. This amount of acetate-carbon in the bicarbonate pool is responsible for the small, but significant, contribution of $C^{13}$ and $C^{14}$ in the carboxyls of alanine, aspartic acid and the α-carboxyl of glutamic acid in series I.

With regard to the reactions, whereby the methyl and the carboxyl of the acetate are metabolized to $CO_2$ under the anaerobic condition in the experiment, we cannot make any definite statements as yet. The outline of a theory will be given in connection with the publication of the isotope distribution in serine and glycine from series I and II.

The over-all picture of the anaerobic metabolism *Rhodospirillum rubrum* as compared with the aerobic photosynthetic mechanism in green plants in general, shows that $CO_2$-fixation results in the formation of relatively few units of the carbon skeleton of the amino acids formed, i. e. some carboxyls. The non-carboxylic parts are almost non-labelled from the $C^{14}O_2$ of series II. The acetate, on the contrary, is responsible for the formation of the other carbon atoms of the amino acids. It seems that the main difference between *Rhodospirillum* under anaerobic condition and green plants in general is that *no main pathway exists in Rhodospirillum from $CO_2$ to the methyl group of acetate*, and that the presence of acetate in the medium consequently is neccessary for anaerobic growth. Other differences of the anaerobic growth of purple bacteria in general and the metabolic system of growing green plants have been discussed by *Franck* and *Gaffron* (14).

## SUMMARY

Alanine, aspartic acid and glutamic acid have been isolated from *Rhodospirillum rubrum*, grown in two series under anaerobic condition under exposure to light, on acetate and bicarbonate as the main sources of carbon. In one of the series the acetate was labelled as $C^{13}H_3C^{14}OONa$, the bicarbonate being non-labelled; in the other series the acetate was non-labelled and the bicarbonate labelled as $NaHC^{14}O_3$. The chemical composition of the media of the two series was identical.

By degradation of the amino acids of both series the isotope content in different carbon atoms was determined.

It was found that carbon-dioxide had entered into the carboxyl of alanine, both carboxyls of aspartic acid and the α-carboxyl of glutamic acid. An insignificant amount of bicarbonate carbon had entered into the non-carboxylic parts of the amino acids isolated.

The carboxyl of the labelled acetate was responsible for the α-carbon atom of alanine and aspartic acid, the γ-carboxyl of glutamic acid, and probably one of its non-carboxylic carbon atoms.

The methyl group of acetate had entered into the β-carbon atom of alanine and aspartic acid and at least two of the middle carbon atoms of glutamic acid.

The acetate recovered from the medium containing $C^{14}$-labelled bicarbonate had a very small isotope content. The bicarbonate from the medium with labelled acetate contained at the end of the growth experiment a small amount

of $C^{13}$ and $C^{14}$, indicating that some amount of the acetate had been metabolized to $CO_2$.

Som aspects of the utilization of acetate under anaerobic condition for growth of *Rhodospirillum* have been discussed. It is assumed that during photoassimilation of acetate, the fixation of $CO_2$ is a secondary process and that the principal part of the photosynthetic mechanism is the conversion of acetate to a reactive 2-carbon compound, which could add $CO_2$ to a 3-carbon compound, closely connected with pyruvic acid and alanine. The latter view is in accordance with the theory of Calvin with respect to the first steps of photoassimilation of $CO_2$ in green plants.

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LITERATURE. 1. **J. Baddiley, G. Ehrensvärd, R. Johansson, L. Reio, E. Saluste** and **R. Stjernholm,** J. Biol. Chem. 183, 771 (1950). — 2. **J. Baddiley, G. Ehrensvärd, E. Klein, L. Reio** and **E. Saluste,** J. Biol. Chem. 183, 777 (1950). — 3. **G. Ehrensvärd, L. Reio** and **E. Saluste,** Acta Chem. Scand. 3, 645 (1949). — 4. **G. Ehrensvärd, L. Reio, E. Saluste** and **R. Stjernholm,** J. Biol. Chem. 189, 93 (1951) — **C. Cutinelli, G. Ehrensvärd, L. Reio, E. Saluste** and **R. Stjernholm,** Acta Chem. Scand. 5, 353 (1951). — **C. Cutinelli, G. Ehrensvärd** and **L. Reio,** Ark. Kemi 2, Nr 21, 357 (1950). — 7. **C. B. van Niel,** Bacteriol. Review 8, 1 (1944). — **M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert** and **P. F. Yankewich** Isotopic Carbon, Wiley, New York (1949) p. 155. — 9. **M. Calvin,** J. Chem. Ed. 26, 639 (1949). — 10. **M. Calvin,** Abstracts of communications, Ith International Congress of Biochemistry, Cambridge (1949) p. 518. — 11. **C. H. Werkman** and **H. G. Wood,** Adv. of Enzymology Vol. II, Interscience Publishers Inc. New York (1942) p. 147. — 12. **N. Lifson, V. Lorber, W. Sakami** and **H. G. Wood,** J. Biol. Chem. 176, 1263 (1948). — 13. **M. D. Kamen, S. J. Ajl, L. S. Ranson,** and **J. M. Siegel** manuscript in press (1951). — 14. **J. Franc** and **H. Gaffron,** Adv. of Enzymology Vol. I, Interscience Publishers Inc. New York (1941) p. 237.

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# Insoluble saccharase in baker's yeast

By Walborg Thorsell and Karl Myrbäck

The saccharase of yeast, especially that of baker's yeast, is more or less firmly bound to insoluble cell constituents (1). Thus plasmolysis of the cells by means of ethyl acetate, toluene, chloroform etc. yields a juice, generally containing only a relatively small part of the saccharase of the yeast. Likewise, extraction of dried baker's yeast or acetone yeast with water gives a very poor yield of soluble saccharase. Prolonged autolysis of plasmolyzed cells or dried yeast with water in presence of toluene slowly brings the enzyme into solution; the process being an enzymatic cleavage of the insoluble enzyme complex. Nothing much is known about the nature of this complex. The protein nature of saccharase can not, of course, be doubted (2). However, the enzyme protein could possibly be united in the cell to insoluble carbohydrates or to insoluble proteins, or the enzyme protein could contain a carbohydrate part, which might be united to other, insoluble carbohydrates. In this connection, it may be mentioned that experiments on the inactivation of saccharase with certain reagents tend to show the existence of a carbohydrate group in the enzyme molecule (3).

Since the liberation of saccharase from the insoluble cell remnants is enhanced by certain enzyme preparations it should be possible, to some degree, to ascertain the nature of the bond uniting the enzyme and the other component(s) of the insoluble complex.

It is well known that the yeast saccharase in the living cell exhibits its full activity when the cells are suspended in sucrose solution. The same activity is found, if fermentation is stopped by addition of toluene etc., and likewise, after complete autolysis when the enzyme is in true solution. Willstätter (4) considered the enzyme to be an "endo-enzyme", not very firmly bound to cell structures, but prevented by the cellular membrane to leave the cell. He regarded the liberation of the enzyme as due to destruction of the membrane, thought to be effected not only by enzymes, but also by mechanical means. However, at least in the case of baker's yeast, it is certainly not possible to liberate the saccharase by mechanical destruction of the cell membrane.

The fermentation of sucrose and other fermentable sugars by living yeast is completely inhibited by toluene. On the other hand, fermentation by yeast juices and such dried yeasts, the membrane of which has been destroyed by the drying[1], is not inhibited by toluene. It seems permissible to assume, that the inhibition of the fermentation by living yeast, is due to an action of the inhibiting substance on the membrane whereby the diffusion of the sugar into the cell is stopped. Since the saccharase action of yeast is not in any way impaired by toluene, it seems safe to conclude that in the saccharase action of the living cell diffusion of the substrate through the membrane plays no role. In other words, it may be assumed that the saccharase is located in or on the cell membrane. To the same conclusion have WILKES and PALMER arrived (5); these authors point out that the enzyme in the cell and purified enzyme in solution have precisely the same activity-pH-curve. In contradistinction to this, the fermentation of yeast juices or dried yeast with destroyed membrane has a narrow pH-optimum between 6.0 and 6.5, whereas living yeast shows a very broad optimum between pH = 2.5 and about 8 (6). Evidently the living cell is able to regulate the pH in its interior, independent to a certain degree of the pH of the surrounding medium.

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[1] The destruction of the membrane is shown, for instance, by the fact that codehydrogenase I, codecarboxylase etc. can be removed from such yeasts simply by washing with water.

These facts tend to show that the saccharase of the cell is firmly bound to the membrane, and that it is not only hindered by it to diffuse out into the solution. The enzyme is liberated, not when the membrane is burst open mechanically or destroyed by enzyme action, but when the enzyme is split off enzymatically from the substance of the membrane to which it is united in the cell.

Other cases are well known where an enzyme in its natural surroundings is bound to some insoluble material from which it must be liberated enzymatically. One example is the β-amylase of barley and certain other cereals (7). In these materials part of the enzyme is readily soluble in water, but the greater part is quite insoluble and can be liberated only by the action of certain proteolytic enzymes of the papain type (8). It should be noted, that the insoluble, "latent" amylase of the cereals is quite *inactive* in its insoluble form whereas the insoluble saccharase of baker's yeast exhibits its full activity. This implies that in the case of β-amylase substrate-binding or otherwise for the enzyme action indispensable groups are involved in the fixation of the enzyme on the insoluble material, but that the insoluble saccharase has all its essential groups free. It is of interest in this connection, that saccharase absorbed on aluminum hydroxide, may exhibit full activity without being eluated (9). Furthermore we have been able to show that saccharase absorbed on Amberlite IRC 50 retains its full activity when brought into sucrose solution. No elution takes place.

WILLSTÄTTER and RACKE (4) treated *brewer's* yeast with ethyl acetate at 37°, thereby inactivating the yeast enzymes capable of liberating saccharase during autolysis. The authors found that the cell remnants could be treated with pepsin and trypsin without liberation of saccharase. Large amounts of inactive proteins and other substances, however, were brought into solution. They found also that saccharase could be liberated by means of malt extract and a preparation of mold "tannase", and concluded that saccharase was set free by carbohydrase action, possibly on yeast gum. However, amylases have no action on yeast gum or other mannans.

After Mr LEO WALLERSTEIN had pointed out that papain is effective in the preparation of soluble yeast saccharase, WILLSTÄTTER and GRASSMANN (10) investigated the papain action. They confirmed the findings of Wallerstein and found, among other things, that hydrocyanic acid increased the capacity of papain to liberate saccharase. It was also found that purified yeast proteinases had a similar action. The question arose whether the action of malt amylase preparations etc. should be attributed to the presence of proteinases.

However, GRASSMANN and PETERS (11) arrived at the conclusion that malt amylase preparations, free from proteinase, are effective. It was believed that the insoluble saccharase exists as an enzyme-protein-carbohydrate "symplex", and that the enzyme could be liberated not only by papain and similar proteolytic enzymes, but also by amylases.

However, several facts make the above conclusions appear questionable. Certainly there are in malt extracts proteolytic enzymes of a papain-like type (they are activated by HCN etc.), but it may be asked if all proteolytic enzymes in the extracts must show their presence in the WILLSTÄTTER-GRASSMANN (12) test. Furthermore, if there are papain-like enzymes in the malt extract (and among the proteolytic enzymes of the yeast), it is difficult to understand why in the experiments of GRASSMANN and PETERS no activation by HCN was found. The authors themselves point out that the commercial amylase preparation "Diamalt" displayed considerably less activity than could be expected with regard to the amylase content of the preparation. Finally, all authors agree that the animal amylases are devoid of any action. By way of explanation GRASSMANN and PETERS mention that, according to investigations by KUHN (13), the "malt amylase" belongs to the β-series of amylases, whereas the animal amylases are α-amylases. If this explanation were correct, one would have to assume that the β-amylase, present in malt extracts together with malt α-amylase, is the enzyme responsible for the liberation of saccharase. In the light of our present knowledge concerning the mode of action of the different types of amylases (7) it appears highly improbable that β-amylase could have such an action. If the amylases in malt extracts have something to do with the liberation of saccharase, it seems necessary to assume that the α-amylase is responsible and, since all α-amylases have practically the same action on their substrates, it is difficult to believe that in this special case the malt α-amylase and the mold α-amylases, which latter do not contain β-amylase, would be active but the animal amylases quite inactive.

GRASSMANN and PETERS mention the possibility that carbohydrases other than the amylases may be thought to play a role in the liberation of saccharase. However, they were able to show, that cellulase which was present in small and strongly varying amounts in the malt extracts, is not involved.

In connection with experiments on the purification of saccharase and its inhibition by certain reagents etc., we have had ample opportunity to make observations concerning the nature of the reaction by which the insoluble saccharase of baker's yeast is brought into solution. Most of our results agree with those quoted above. Thus we confirmed the statement, that papain has a strong action, but trypsin practically none. Furthermore we found the animal amylases quite inactive, whereas malt and mold amylase preparations exhibited a more or less pronounced activity. In certain respects, however, our experimental results differed from those of WILLSTÄTTER and GRASSMANN: We found that β-amylase (barley extract) with a saacharifying power exceeding that of a malt extract had, compared to the malt extract, only a slight action on the insoluble saccharase, an action which doubtlessly is to be explained as due to the barley proteases. We were also able to show that certain purified preparations of "malt amylase", containing very large amounts of β- and α-amylase had no action at all on the liberation of saccharase. Finally we found, that the action of raw malt extracts is enhanced by hydrogen sulfide in the same way as papain.

## Experimental part

*Preparation of "insoluble saccharase".* 1 kg of pressed yeast (dry weight 25 %) was mixed thoroughly with 200 ml of neutral ethyl acetate and the mixture was stirred mechanically at room temperature for two hours. The slurry was diluted with several liters of water and centrifuged. The cell remnants were washed repeatedly in the centrifuge with large amounts of water until the smell of ethyl acetate had vanished. The insoluble residue was diluted with 1000 ml of water, and toluene was added. The suspension contained nearly all saccharase of the original living yeast but a filtrate was quite devoid of action on sucrose. Even after weeks at 30° only very small amounts of saccharase had passed into solution. This shows that the enzymes effecting the liberation of saccharase during prolonged autolysis, *i.e.* certain proteolytic enzymes of the yeast, had been effectively removed (or possibly inactivated) during plasmolysis and washing. Willstätter and Grassmann inactivated these enzymes by treatment of the yeast with ethyl acetate at 37°.

*Action of β- and α-amylases.* By pointing out that the "malt amylase" is a β-amylase, GRASSMANN had, tentatively, explained the fact that malt amylase, but not the animal amylases are setting the bound saccharase free. We have, therefore, compared the two types of amylases. At the present time we know (7) that "malt amylase" is a mixture of β-amylase and α-amylase. The latter is formed during germination, the former is present already in the non-germinated barley which contains, at most, only traces of α-amylase. To the following experiments (Table 1) extracts of barley and malt were used that had practically the same saccharifying action on starch. The barley amylase was free from α-amylase. 50 ml suspension of insoluble saccharase was mixed with 5 ml enzyme extract. pH was adjusted to about 5.3. The mixtures were held at 30°. Samples were removed after the times mentioned in the table and filtered. In the filtrates saccharase was determined in the usual way polarimetrically (14).

*Table 1*

| Enzyme added | % saccharase liberated after | | |
|---|---|---|---|
| | 24 hrs | 115 hrs | 360 hrs |
| No enzyme | — | 1.8 | 2.8 |
| Barley extract (β-amylase) | 7.7 | 18.1 | 46.2 |
| Malt extract (α- and β-amylase) | 20.5 | 44.5 | 90.3 |

It must be concluded that the much higher effectivity of malt extract is due either to α-amylase or to the increase of proteolytic activity during germination of the barley.

*Action of malt extract and certain proteolytic enzymes.* The effect of the following preparations was investigated in the same way as above (Table 2).

1) Papain, commercial preparation 1 : 350. 1 g papain was dissolved in 50 ml suspension and the pH was adjusted to 5.1. The papain preparation had no action on sucrose or starch paste.

2) Crystalline trypsin (Armour Co.); 0.03 g to 50 ml suspension; pH = 7.0. The enzyme of course had no action on sucrose or starch.

3) Pancreatic trypsin preparation (Fine Chemical Co., N.Y.); 0.75 g to 50 ml suspension; pH = 7.0. The enzyme preparation had no action on sucrose but contained large amounts of α-amylase.

4) Malt extract 1 : 4 (malt made from six-row barley); 100 ml to 50 ml suspension; pH = 5.3. The malt extract did not split sucrose but contained large amounts of β- and α-amylase, presumably also several other enzymes, among them proteases.

5) Purified preparation (15) of "malt amylase" *i.e.* a mixture of α- and β-amylase as in the malt itself. The amount used had an action on starch comparable to that of the malt extract used in experiment 4). The degree of purity (on a dry weight basis) was at least 100 times that of the malt extract. pH = 5.0. *The preparation did not contain proteolytic enzymes.*

Parallel experiments with the same pH-values but without addition of foreign enzymes were performed in each case. Temperature 30°. After the times mentioned in table 2 aliquots of the suspensions were filtered and the saccharase assay carried out as above.

*Table 2*

| Enzyme | Time | % saccharase liberated | |
|---|---|---|---|
| | | with enzyme | without enzyme |
| 1) Papain | 11 days | 53 | < 7 |
| 2) Crystalline trypsin | 4 ” | 7 | ~ 2 |
| 3) Trypsin (Fine Chem. Co.) | 6 ” | 8 | ~ 3 |
| 4) Malt extract | 4 ” | 68 | ~ 3 |
| 5) Purified malt amylases | 4 ” | ~ 2 | ~ 2 |

Saccharase determination *in the suspension* showed that no perceptible inactivation of the saccharase had taken place. Experiments with suitable substrates demonstrated that the enzymes used for liberating the saccharase were still active after the times mentioned in table 2. The saccharase determinations in the filtrates tend to show that not only the animal amylases, but also those of malt are quite without any action on the insoluble saccharase complex. Trypsin has only a very slight action, possibly because the pH used was not optimal for trypsin action. No difference was found between crystalline trypsin and raw trypsin containing large amounts of amylase (and lipase). The papain preparation and the raw malt extract had a pronounced action.

Similar experiments were carried out with the following enzyme preparations, using another batch of insoluble saccharase.

1) No enzyme added; pH = 5.5.
2) Pancreatin (Ph. Suec. Ed. X); 0.5 g in 50 ml suspension; pH = 6.
3) Papain Merck 1 : 150; 0.3 g in 50 ml suspension; pH = 5.5.
4) Diastase Merck (Ph. Jap. IV); 0.5 g in 50 ml suspension; pH = 5.5.
5) Malt extract 1 : 4; 5 ml to 50 ml suspension; pH = 5.3.
6) Enzymes of 25 ml malt extract, precipitated with tannin and redissolved; 50 ml suspension; pH = 5.3.

7) Enzymes of 25 ml malt extract heated to 70° for 15 minutes, then precipitated with tannin etc.; 50 ml suspension; pH = 5.3.

8) 5 ml saliva; 50 ml suspension; pH = 6.5.

The liberation of saccharase is shown in table 3.

*Table 3*

| Enzyme | % saccharase liberated after | | | |
|---|---|---|---|---|
| | 3 days | 7 days | 8 days | 11 days |
| 1) No enzyme | 7 | — | 11 | 12 |
| 2) Pancreatin | 10 | — | — | 13 |
| 3) Papain | 25 | — | — | 84 |
| 4) Diastase Merck | 48 | — | — | 100 |
| 5) Malt extract | — | 55 | — | — |
| 6) Malt extr. precipitated | — | — | 90 | — |
| 7) Malt extr., heated and prec. | — | — | 25 | — |
| 8) Saliva | — | — | 12 | — |

Pancreatin and saliva have no action at all. The various amylase preparations from malt and moulds on the other hand, show a more or less strong action on the saccharase. These preparations, however, contain proteases of the papain type and other enzymes in addition to the amylases. In order to further elucidate the nature of the enzyme responsible for the saccharase liberation, the action of hydrogen sulfide on the reaction was studied: 50 ml suspension + 5 ml 10 % papain solution or 5 ml malt extract or 5 ml water. In parallel experiments the enzyme solutions were saturated with hydrogen sulfide before the addition to the insoluble saccharase suspension. Results in table 4.

*Table 4*

| Enzyme | % saccharase liberated after | |
|---|---|---|
| | 24 hrs | 115 hrs |
| 1) Suspension alone | — | 1.8 |
| 2) " " + $H_2S$ | 4.0 | 7.0 |
| 3) Malt extract | 20.5 | 44.5 |
| 4) " " + $H_2S$ | 37.1 | 56.8 |
| 5) Papain | 44.8 | 79.8 |
| 7) " + $H_2S$ | 94.0 | 100 |

In all cases the liberation of saccharase is greatly enhanced by hydrogen sulfide.

## SUMMARY

The liberation of the saccharase of baker's yeast from its insoluble complex is effected by proteolytic enzymes of the papain type. Amylases of animal or plant origin have no action, provided the preparations are free from papain-

like enzymes. Trypsin seems to have only a very slight if any action. It seems permissible to assume that the insoluble saccharase is a complex of the soluble enzyme and some insoluble protein. The insoluble complex has approximately the same saccharase activity as the soluble enzyme.

Perhaps it would be as well to regard the insoluble "complex" as the original enzyme and to assume that part of the enzyme protein can be, without abolition of the enzymatic activity, split off by certain proteases, thereby rendering the active part of the molecule soluble in water. In a way the case is an analogue to that of chymotrypsin; parts of the original (soluble) chymotrypsin protein molecule can be split off and new chymotrypsins with the same specificity and activity are formed (16).

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REFERENCES: 1) **Kraut, H.** and **Rohdewald, M.,** *Carbohydrasen* in **G. M. Schwab:** *Handbuch der Katalyse* III, *Biokatalyse.* Springer-Verlag, Wien 1941, p. 66. — 2) Survey of modern investigations on saccharase: **Neuberg, C.** and **Mandl, I.,** *Invertase* in **Sumner, J. B.** and **Myrbäck, K.,** *The Enzymes, Chemistry and Mechanism of Action,* Academic Press Inc. New York 1950, Vol. I, p. 527. — 3) **Myrbäck, K.,** Z. physiol. Chem. *158,* 160 (1926). — 4) **Willstätter, R.** and **Racke, F.,** Ann. *425,* 1 (1921); *427,* 111 (1921). **Willstätter, R.** and **Rohdewald, M.,** Z. physiol. Chem. *209,* 38 (1932). — 5) **Wilkes, B. G.** and **Palmer, E. T.,** J. Gen. Physiol. *16,* 233 (1932). — 6) **Myrbäck, K.,** Z. physiol. Chem. *139,* 30 (1924). — 7) **Myrbäck, K.** and **Neumüller, G.,** *Amylases and the hydrolysis of starch and glycogen,* in **Sumner, J. B.** and **Myrbäck, K.,** *The Enzymes,* Vol. 1, p. 653. — 8) **Myrbäck, K.** and **Örtenblad, B.,** Enzymologia *2,* 305 (1937). — 9) **v. Euler, H.** and **Myrbäck, K.,** Z. physiol. Chem. *127,* 115 (1923). — 10) **Willstätter, R.** and **Grassmann, W.,** Biochem. Z. *203,* 308 (1928). — 11) **Grassmann, W.** and **Peters, T.,** Z. physiol. Chem. *204,* 135 (1932). — 12) **Willstätter, R.** and **Grassmann, W.,** Z. physiol. Chem. *138,* 184 (1924). — 13) **Kuhn, R.,** Ann. *443,* 1 (1925). — 14) **Weidenhagen, R.,** in **Bamann, E.** und **Myrbäck, K.,** *Die Methoden der Fermentforschung,* G. Thieme, Leipzig, 1941, p. 1725. — 15) **Holmbergh, O.,** Svensk kem. tid. *62,* 1 (1950); Arkiv Kemi, *2,* 125 (1950). — 16) **Smith, E. L.,** in **Sumner, J. B.** and **Myrbäck, K.,** *The Enzymes,* Vol. I, Part 2, p. 849.

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# Optically active thiophene derivatives. III. Experiments on optical resolution of phenyl-α-thienyl-glycolic acid and benzyl-α-thenyl-acetic acid

By Arne Fredga, Kaj Aejmelaeus and Bengt Tollander

With 3 figures in the text

In order to compare the influence of the phenyl and the thienyl group on the rotatory power of optically active compounds, we have tried to prepare and resolve compounds containing both groups as asymmetry elements, attached to the same asymmetric carbon atom in the same manner.

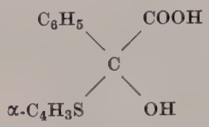
In some cases, thiophene compounds have been reported to be isomorphous or at least crystallographically related to the corresponding benzene derivatives (1, 2, 3, 4). If two of the groups attached to an asymmetric carbon atom are able to replace each other isomorphously, there may be a certain possibility that the optical antipodes of the compound in question are isomorphous. If the compound is an acid, the same may hold good for the salts of the antipodes with a given alkaloid. Even if the salts are not isomorphous, they can be expected to have rather similar physical properties. If this is so, the prospects of separating the antipodes are not encouraging.

The phenyl-α-thienyl-glycolic (α-thienyl-mandelic) acid (I) can be prepared in different ways. The ethyl ester was described by Steinkopf and Hanske (5) more than ten years ago and the free acid was first prepared by Blicke and Tsao (6).

Resolution experiments with different bases gave optically active acid, but the rotatory power was, in most cases, very low. The best results were obtained with α-phenylethylamine. Repeated recrystallisations of the salts with dextrorotatory and levorotatory amine yielded acids with $[\alpha]_D = -28^\circ$ and $+25^\circ$ respectively. The levorotatory acid may possibly represent the pure (—)-form. It is thus possible to resolve practically a compound having both a phenyl and a thienyl group attached to the asymmetric carbon atom. To our knowledge, this is the first instance of an optically active compound of this type.

The acid is, however, rather sensitive to light and chemical influence. It easily assumes a brown colour, after which it is very difficult to purify. The yield of active acid was also very low. The compound is thus unsuitable for precise optical activity measurements so the investigation was not pursued further.

The benzyl-α-thenyl-acetic acid (II) is obtained upon condensation of α-thenyl chloride with ethyl benzylmalonate and subsequent hydrolysis and decarboxyla-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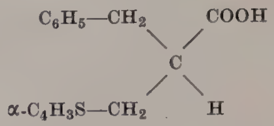
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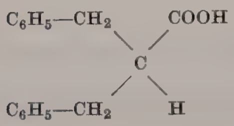
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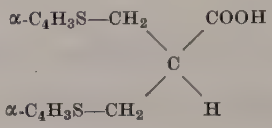
(IV)

tion. It gives readily crystallising salts with organic bases, but in spite of extensive experiments with different alkaloids, no optically active acid could be isolated.

As pointed out above, the optical resolution of a racemic compound can be expected to meet with difficulties if the antipodes are isomorphous. If the inactive benzyl-α-thenyl-acetic acid is a solid solution of the antipodes, it can be expected to give solid solutions with dibenzyl-acetic acid (III) or di-α-thenyl-acetic acid (IV) or both. The melting point diagrams of the different systems were therefore studied (fig. 1–3). They are, however, all of the common eutectic type, and the inactive acids are thus not completely miscible in solid phase. In view of this fact, it seems rather unlikely that the inactive benzyl-α-thenyl-acetic acid is a solid solution of the antipodes.

The failure to obtain optically active acid may, of course, also be due to the extremely low rotatory power of the antipodes. Neither the benzene nor the thiophene nucleus is directly attached to the asymmetric carbon atom, and in this position their influence on the rotatory power must be reduced.

Recent experiments in collaboration with H. EKEMARK indicate, that the phenyl-α-thienyl-acetic can be resolved with α-phenylethylamine. As this acid is rather stable, it may form suitable material for studying the activity of a substance of this type under different conditions.

## Experimental

### Phenyl-α-thienyl-acetic acid

(Experiments in collaboration with K. AEJMELAEUS)

The *ethyl ester* was prepared from ethyl phenylglyoxylate and α-thienyl-magnesium iodide using the method of STEINKOPF and HANSKE (5). After two distillations *in vacuo*, it was recrystallised from light petrol (b.p. 30–50°).

22 g of the ester were hydrolysed with 7 g of sodium hydroxide (80 % excess) dissolved in 30 ml water + 15 ml alcohol. When the alcohol was evaporated, an oil separated, which redissolved when 25 ml of water were added. After cooling, the solution was acidified with an excess of dilute sulphuric acid. The acid, which separated in a crystalline form, was filtered off, dried and recrystallised from benzene. The yield of crude product was 19 g. The pure acid was obtained as long, thin needles, which on heating assume a brown colour at about 107° and melt, decomposing, at about 120°.

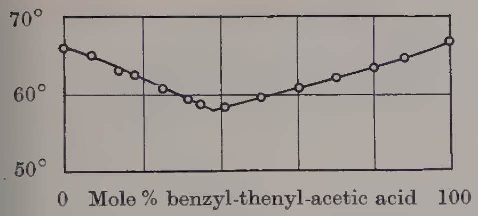

Fig. 1. Benzyl-α-thenyl-acetic acid (II) and di-α-thenyl-acetic acid (IV).

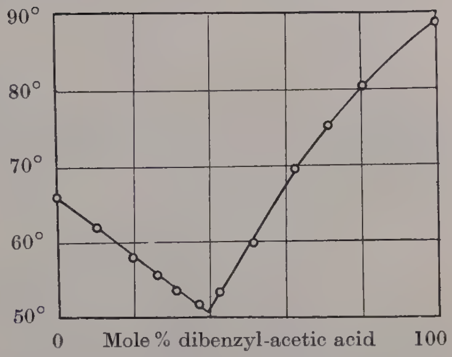

Fig. 3. Dibenzyl-acetic acid (III) and di-α-thenyl-acetic acid (IV).

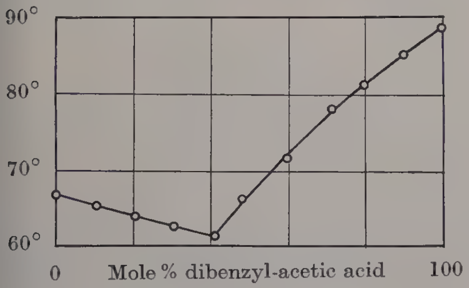

Fig. 2. Benzyl-α-thenyl-acetic acid (II) and dibenzyl-acetic acid (III).

0.1604 g sbst: 4.50 ml 0.1516-*N* NaOH. — 24.46 mg sbst: 55.09 mg $CO_2$ and 9.29 mg $H_2O$. — 27.96 mg sbst: 27.93 mg $BaSO_4$.

| | | | | |
|---|---|---|---|---|
| $C_{12}H_{10}O_3S$ calc. | Equiv. wt. 234.3 | C 61.50 | H 4.31 | S 13.69 |
| found | » » 235.1 | » 61.42 | » 4.25 | » 13.72 |

*Preliminary experiments on resolution.* Equimolar amounts of acid and alkaloid were dissolved in methanol, and water was carefully added. The, more or less, readily crystallising salt was filtered off, the acid was liberated, and its rotatory power determined in ethyl acetate solution. (Table 1).

*Table 1*

| | g acid | g alkaloid | g salt | $[\alpha]_D$ |
|---|---|---|---|---|
| Brucine | 0.235 | 0.394 | 0.293 | $-1°$ |
| Strychnine | 0.235 | 0.334 | 0.218 | $+1°$ |
| Quinine | 0.235 | 0.378 | 0.429 | $+0.5°$ |
| Quinidine | 0.235 | 0.369 | 0.433 | $-1.5°$ |
| Cinchonine | 0.235 | 0.294 | 0.295 | $-4.5°$ |
| Cinchonidine | 0.235 | 0.294 | 0.379 | $-1°$ |
| (+)-Phenylethylamine | 1.85 | 0.920 | 1.602 | $-8.2°$ |

*Experiments with α-phenylethylamine on a larger scale.* 21.9 g acid and 11.3 g (+)-α-phenylethylamine (7) were dissolved in 40 ml methanol, and 40 ml water were added. The resulting salt was recrystallised several times from aqueous (55 %) methanol, using 3.5 ml solvent for each g salt. From each crystallisation, a small sample of the acid was liberated and the activity was determined in ethyl acetate solution.

| Crystallisation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| g salt obtained ................ | 15.7 | 12.7 | 6.35 | 5.80 | 3.60 | 2.63 | 1.65 | 1.00 |
| $[\alpha]_D$ of the acid ............... | −5° | −7° | −12° | −17.5° | −21° | −26° | −28° | −28° |

From the mother liquors remaining after the first crystallisations an acid having $[\alpha]_D = +4.2°$ was isolated. 2.8 g of this acid and 1.4 g (—)-α-phenylethylamine (7) were dissolved in dilute methanol and the resulting salt was recrystallised as before.

| Crystallisation | 1 | 2 | 3 |
|---|---|---|---|
| g salt obtained................ | 2.6 | 1.6 | 0.6 |
| $[\alpha]_D$ of the acid............... | +9.5° | +17° | +25° |

From the activity data for the (—)-acid it seems probable that maximum activity was obtained in this case. The isolation of the antipodes in a state of indisputable purity would, however, require experiments on a still larger scale.

## Benzyl-α-thenylacetic acid

### (Experiments in collaboration with B. TOLLANDER)

*Synthesis of the acid.* 3.5 g sodium were dissolved in about 75 ml absolute alcohol. 37.5 g (0.15 mole) ethyl benzylmalonate and 20.0 g (0.151 mole) α-thenyl chloride (4) were added, and the mixture was refluxed until it was neutral (about two hours). The ester was then hydrolysed by adding 30 g of potassium hydroxide dissolved in the necessary amount of alcohol. The mixture was refluxed for four hours, the major part of the alcohol was evaporated and the residual salt mass dissolved in water. The remaining alcohol was now driven off and the aqueous solution cooled and acidified with hydrochloric acid to pH = 1. An oil separated, which gradually crystallised; in some experiments it was found convenient to inoculate with crystals from a former preparation. A small portion of the reaction product could be obtained by ether extraction of the acid solution.

The crystal mass, a mixture of benzyl-thenyl-malonic and benzyl-thenyl-acetic acid, was heated to 130–135° for two hours, after which the malonic acid was completely decarboxylated. The resulting benzyl-thenyl-acetic acid was decolourised with norite in ethereal solution. After evaporation of the ether, the residue was recrystallised several times from light petrol (b.p. 30–50°). The yield was 25 g (67 %). The acid, which can also be crystallised from ligroin (b.p. 60–70°), forms short-prismatic crystals with m.p. 66.8°.

0.46925 g sbst: 19.07 ml 0.09977-*N* NaOH. — 20.94 mg sbst: 52.39 mg $CO_2$ and 10.46 mg $H_2O$. — 36.09 mg sbst: 34.00 mg $BaSO_4$.

| | | | | |
|---|---|---|---|---|
| $C_{14}H_{14}O_2S$ calc. | Equiv. wt. 246.3 | C 68.26 | H 5.73 | S 13.02 |
| found | » » 246.6 | » 68.28 | » 5.59 | » 12.94 |

*Experiments on resolution.* 0.246 g (0.001 mole) acid and 0.001 mole alkaloid were dissolved together in 5 ml methanol and water was added just to turbidity. The yields of crystallised salt from the different alkaloids are tabulated below; the salts in the first row crystallised rather easily, those in the second only with difficulty

| | | | |
|---|---|---|---|
| Cinchonine | 0.403 g | Brucine | 0.531 g |
| Cinchonidine | 0.315 g | Strychnine | 0.460 g |
| Quinine | 0.321 g | Quinidine | 0.446 g |
| Ephedrine | 0.190 g | Morphine | 0.441 g |
| α-Phenylethylamine | 0.312 g | | |

The acid liberated from the salts was in all cases optically inactive. No crystallised salts could be obtained from sparteine and nicotine.

Several attempts were made to recrystallise the salts from organic solvents, but in most cases their solubility was too high. The salts obtained from cinchonine, quinine, morphine and α-phenylethylamine could, however, be recrystallised from ethyl acetate. The morphine salt could also be recrystallised from chloroform or acetone and the cinchonine salt from acetone. The acid obtained from the recrystallised salts was in all cases completely inactive.

*Synthesis of di-α-thenyl-acetic acid.* 3.5 g sodium were dissolved in absolute alcohol and 24 g (0.15 mole) diethyl malonate were added. After cooling, 20.0 g (0.151 mole) α-thenyl chloride were added and the mixture was refluxed until the reaction was neutral. A solution of sodium ethylate, prepared by dissolving another 3.5 g of sodium in absolute alcohol was now added and the mixture was allowed to cool. After another 20.0 g of α-thenyl chloride were added, the mixture was refluxed once more until it was neutral. The hydrolysis and decarboxylation of the malonic acid were carried out as described before, and the product was recrystallised from light petrol or ligroine. The yield was 23.5 g (61 %). The acid forms short-prismatic crystals with m.p. 66.0°. It is rather sensitive to light.

0.44434 g sbst: 17.68 ml 0.09977-$N$ NaOH. — 22.43 mg sbst: 47.06 mg $CO_2$ and 9.64 mg $H_2O$. — 40.17 mg sbst: 6.354 ml 0.1000-$N$ NaOH.

| | | | | | |
|---|---|---|---|---|---|
| $C_{12}H_{12}O_2S_2$ | calc. Equiv. wt. | 252.3 | C 57.11 | H 4.79 | S 25.41 |
| | found » » | 251,9 | » 57.25 | » 4.81 | » 25.31 |

The *dibenzyl-acetic acid* was prepared using the method of Bischoff and Siebert (8). We found the m.p. 88.7°; the literature gives values between 85 and 90°.

The melting points for the phase diagrams were determined by Rheinboldt's method (9) using an apparatus desribed by Friedrichs (10). The results for the three systems investigated (II and IV, II and III, III and IV) are given in tables 2–4 and figures 1–3.

Part of the expenses involved in this research has been defrayed by a grant from the Nobel Fund of the Royal Swedish Academy of Science (Kemiska prisgruppens särskilda fond).

*Table 2*

| II and IV | |
|---|---|
| Mole % of II | M.p. |
| 0.0 | 66.0° |
| 7.1 | 65.0° |
| 14.0 | 63.1° |
| 18.1 | 62.5° |
| 25.3 | 60.8° |
| 31.9 | 59.4° |
| 35.1 | 58.7° |
| 41.4 | 58.4° |
| 50.8 | 59.7° |
| 60.7 | 60.8° |
| 70.3 | 62.1° |
| 80.1 | 63.4° |
| 88.1 | 64.7° |
| 100.0 | 66.8° |

*Table 3*

| II and III | |
|---|---|
| Mole % of III | M.p. |
| 0.0 | 66.8° |
| 10.5 | 65.4° |
| 20.4 | 64.0° |
| 30.2 | 62.6° |
| 41.0 | 61.3° |
| 48.1 | 66.1° |
| 59.8 | 72.6° |
| 71.2 | 78.0° |
| 79.8 | 81.2° |
| 90.0 | 85.2° |
| 100.0 | 88.7° |

*Table 4*

| III and IV | |
|---|---|
| Mole % of III | M.p. |
| 0.0 | 66.0° |
| 10.3 | 62.0° |
| 19.9 | 58.0° |
| 26.0 | 55.7° |
| 31.0 | 53.6° |
| 37.0 | 51.7° |
| 42.3 | 53.4° |
| 51.6 | 59.8° |
| 62.7 | 69.5° |
| 71.3 | 75.2° |
| 80.5 | 80.4° |
| 100.0 | 88.7° |

## Summary

Racemic phenyl-α-thienyl-glycolic acid (I) has been resolved, with active α-phenylethylamine, into samples having $[\alpha]_D = +25°$ and $-28°$ respectively. This is, to our knowledge, the first instance of an optically active compound with both a phenyl and a thienyl group attached to the same asymmetric carbon atom.

Benzyl-α-thenyl-acetic acid (II) has been synthesised, but attempts to resolve it into the antipodes have, as yet, proved fruitless. Possible explanations of the failure are discussed.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, March 1951.

REFERENCES. (1) **G. Bruni** and **G. Natta,** Rec. trav. chim. *48,* 860 (1929). — (2) **C. Liebermann,** Ber. *26,* 853 (1893). — (3) **K. Mislow,** J. Phys. & Colloid Chem. *52,* 729 (1948). — (4) **A. Fredga** and **O. Palm,** Arkiv Kemi, Mineral. Geol. *26 A* N:o 26 (1949). — (5) **W. Steinkopf** and **W. Hanske,** Ann. *541,* 253 (1939). — (6) **F. F. Blicke** and **M. U. Tsao,** J. Am. Chem. Soc. *66,* 1645 (1944). — (7) Org. Syntheses *17,* 80 (1937). — (8) **C. A. Bischoff** and **H. Siebert,** Ann. *239,* 92 (1887). — (9) **H. Rheinboldt,** J. prakt. Chem. [2] *111,* 242 (1925). — (10) **F. Friedrichs,** Z. angew. Chem. *34,* 61 (1921).

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# A study in continuous zone electrophoresis in filter paper.

By Inger Brattsten and Anna Nilsson

With 3 figures in the text

Some orienting experiments of carrying out continuous electrophoretic separations of dyes and coloured algae proteins in sheets of filter paper by the method of cross-wise electrical and liquid flow fields (1, 2. 3) were mentioned by Svensson and Brattsten (2). In these experiments, the upper edge of the paper dipped into a buffer container, except for a fringe through which the dye solution was sucked up into the paper. After passing through the paper, the liquid was collected from fringes cut out in the paper along the lower edge. Platinum electrodes were attached at the two vertical edges of the paper. A similar arrangement is used in an apparatus recently described by Grassman and Hannig (4), which is reported to give good resolution of amino acid and protein mixtures in buffered solutions. Some further results with this technique are reported here.

In the construction of an apparatus for continuous electrophoretic separation in filter paper much the same factors are essential as when a glass powder apparatus is concerned. In addition, it is especially important in the filter paper technique to avoid a free liquid layer on top of the paper in direct contact with it. Due to the high resistance in the paper, part of the electric current would otherwise pass through the free liquid instead of through the paper. The evaporation of water from the paper surfaces should be reduced as much as possible. In filter paper electrophoresis this requirement has been met with in different ways. Immersing the paper into a non-conducting liquid was used by Cremer and Tiselius (5), enclosing it in a moist chamber by Wieland and Fischer (6), Durrum (7), Turba and Enenkel (8), and Grassman and Hannig (4), and clamping it between glass plates by Durrum[1] and Kunkel and Tiselius (9). The control of the electrode reactions requires special consideration. In general it is necessary to arrange a faster flow of the electrode liquid than of the buffer in the middle section.

**Description of the apparatus.** The present apparatus is sketched in Fig. 1. The dimensions of the paper are 40 × 20 cm., except the fringes. It is enclosed between two plates of mirror glass of 7 mm. thickness. To decrease the adhesion of liquid to the glass walls, the plates are treated with silicone grease before being put in contact with the moist paper. Outside the glass plates cooling mantles of lucite are attached for circulating water. The cooling mantles and

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[1] E. L. Durrum, personal communication (*cf.* ref. 2 p. 411).

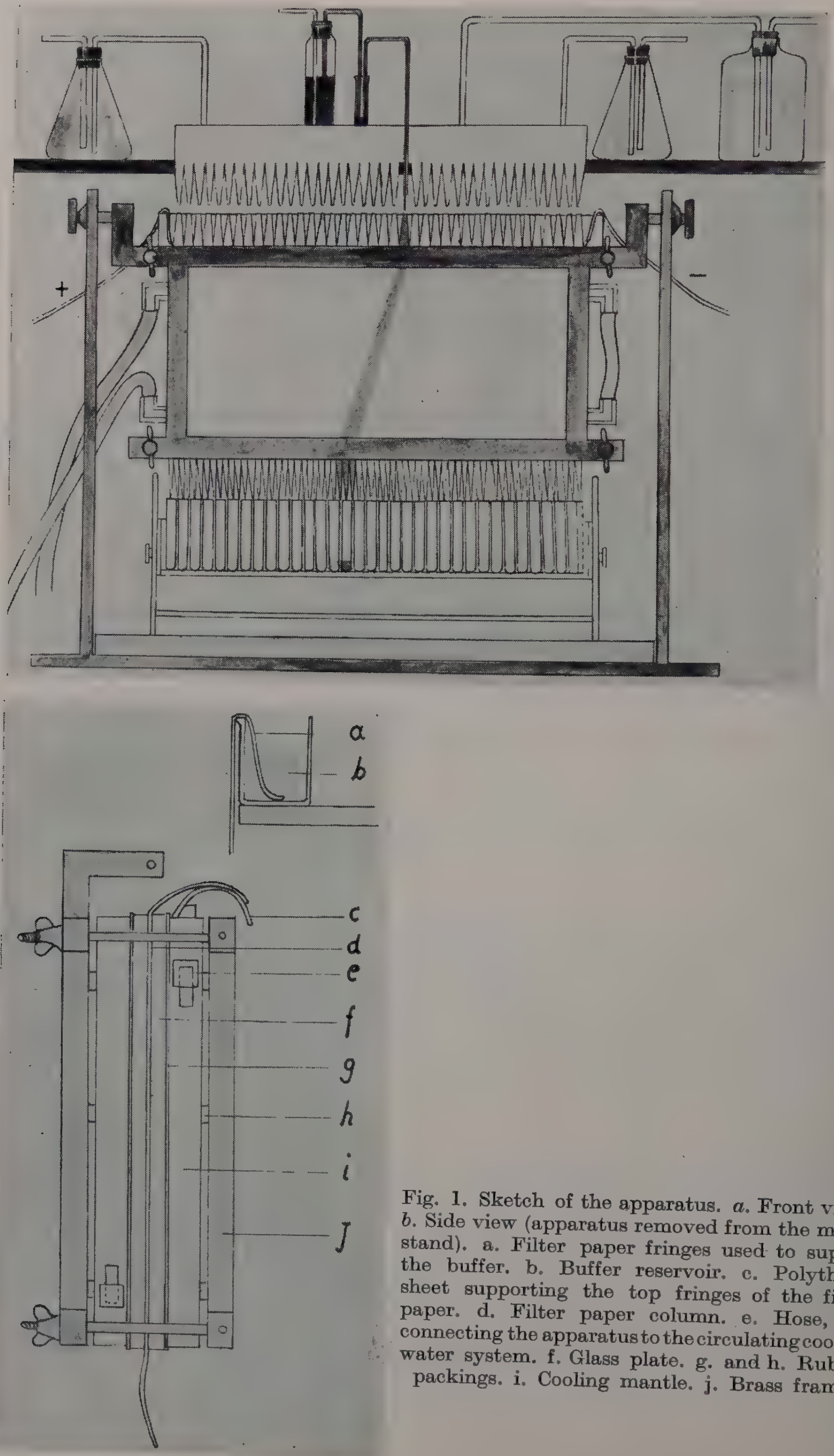

Fig. 1. Sketch of the apparatus. *a.* Front view. *b.* Side view (apparatus removed from the metal stand). a. Filter paper fringes used to supply the buffer. b. Buffer reservoir. c. Polythene sheet supporting the top fringes of the filter paper. d. Filter paper column. e. Hose, for connecting the apparatus to the circulating cooling water system. f. Glass plate. g. and h. Rubber packings. i. Cooling mantle. j. Brass frame.

the glass plates are clamped to the paper by the aid of two brass frames and rubber packings. The pressure of the frames on the plates is regulated by screws at the corners and by varying the heights of the rubber packings.

The liquid flow is arranged in the following way. The top part of the paper is cut into forty fringes, which rest on a polythene sheet. Above each of the fringes, and somewhat behind the apex of the polythene sheet, another set of fringes is placed, which dips into a buffer reservoir. Thus a flow of liquid takes place from the upper fringes to the lower ones, without contact of the paper material. The upper set of fringes is cut from a paper of such quality that the proper rate of flow is obtained. This should be somewhat higher than the velocity through the apparatus, and surplus liquid is allowed to flow down the tips of the fringes and is collected in a container behind the apparatus. The protein mixture is introduced through a piece of polythene cannula from a constant level flask. The adjustment of the breadth of the protein band is made by regulation of the length of the cannula and the hydrostatic pressure. If it is necessary to make the band visible on the paper, some dye is added to the mixture while the rate of flow is regulated. The outflow of the liquid takes place from sixty-six fringes at the lower end of the paper. The fractions are collected in small test tubes. The apparatus is supported by a metal stand and can be tilted around a horizontal axis. Hereby the vertical level difference is changed. To some extent the velocity of flow can be regulated by this means.

Several kinds of paper have been tried with good results. In addition to favourable physico-chemical properties, the paper should be resistant mechanically in the wet condition. It is desirable that one paper should be used in the apparatus for a number of runs. In general, a double layer of the "Munktell OB" paper is satisfactory.

The vertical edges of the glass plates are wedge shaped, thus forming narrow chambers when clamped to the paper. Here the platinum electrodes are placed, and the chambers are closed in the vertical direction by thin plates of lucite. In most instances a concentrated buffer is supplied at the electrodes. In some systems the use of alkali and acid is advantageous to neutralize the electrode reaction products. In highly alkaline or acidic systems no extra electrode liquid is needed.

The electrical power source has the capacity of 250 milliamperes and 1000 volts.

**Experimental.** The experiments performed with this apparatus are test runs and have the main aim of studying the factors which determine the resolution under various conditions. The samples were generally collected during 5 to 10 hours but no attempts have been made to extend the experiments over long periods of time. To obtain some quantitative measure of the separation efficiency, substances of known electrical mobilities were used. The red coloured algae protein phycoerythin was not suitable in this work because it showed too strong adsorption to the paper. Experiments with a mixture of glycine, alanine and glycylglycine in acid medium, and with a mixture of serum albumin and $\gamma$-globulin in a neutral buffer are reported herein.

In the electrophoresis of the amino acids, formate buffer of pH 2.61 and varying ionic strength was used. The amino acid solution contained 0.1 % glycine, 0.1 % alanine and 0.2 % glycylglycine. This solution was introduced into the apparatus as an 1.5 cm broad band. In the outflowing liquid the amino acids were localized by the ninhydrin test. The identity of the substances was established by paper chromatography, and the nitrogen content determined by Kjeldahl analyses. In overlapping parts of the bands, the concentration distribution was estimated from the colour intensity of the ninhydrin spots in the paper chromatograms.

Even in low concentration these substances give rise to measurable conductivity gradients at the zone boundaries, which cause some broadening of the bands during migration. (To a certain extent this effect can be counteracted by keeping a lower ionic strength in the amino acid solution than in the pure buffer.) Electrophoresis experiments were performed at the ionic strengths 0.05, 0.1, and 0.2. At low total salt contents the high mobilities and the possibility of applying a high field strength favour the separation, but the boundary spreading is appreciable. The absolute displacements which can be obtained decrease with increasing ionic strength. The best results were obtained with the ionic strength 0.2. Here the loss in absolute velocity of the bands was compensated by the sharpness of the boundary regions. For comparison, the results of this experiment and one performed at the ionic strength 0.1 are brought together in Fig 2. In the latter of these experiments, B, the broadening of the bands is but slight. Kjeldahl analysis showed that here the concentrations in the centres of the bands were somewhat higher than in the injected solution. This is the result of an initial sharpening of the bands at a vertical conductivity gradient obtained by using a somewhat lower salt content in the amino acid mixture than in the pure buffer.

For glycylglycine a total yield of 1.74 mg per hour is calculated from the nitrogen determinations. If the same quantity is computed with the aid of the concentration of the injected solution, 1.69 mg per hour is obtained. Half as much was introduced per hour of each of the simple amino acids.

Although high wattages were applied in these runs, there were no indications that the temperature rise in the paper was very high. After an initial rise during the first few minutes the current remained stable. In case B of Fig. 2 an electrical effect of 133 watts was used. The initial rise of the current indicated a temperature increase of about 5 degrees. This suggests that a still higher voltage could have been used.

In the protein experiments, a mixture of 0.70 % albumin and 0.56 % γ-globulin in phosphate buffer of pH 7.70 was introduced as 1 cm broad bands. Experiments were performed using varying ionic strength of the buffer. After the passage through the electrical field, the protein components were traced in the fractions by the sulfosalicylic acid test. The concentrations were determined refractometrically or estimated from the turbidity of the samples with the sulfosalicylic acid reagent. Fig. 3 is a representation of two of these experiments, performed at the ionic strengths 0.05 and 0.2. A comparison shows that the greater displacement difference in the former experiment is to some degree conteracted by the greater spreading of the bands, which causes a considerable dilution. The maximum voltage was used in experiment A, whereas in B the efficiency of the separation process was limited by the capacity of the apparatus of neutralizing the electrode reaction products. In case B, the concentration conditions in the bands were quite good. The highest albumin concentration was 0.68 %, and the highest γ-globulin concentration 0.56 %. Four mg protein per hour was introduced in this experiment. This comparatively low value is due to the fact that the filter paper column was thinner in this case than in the experi-

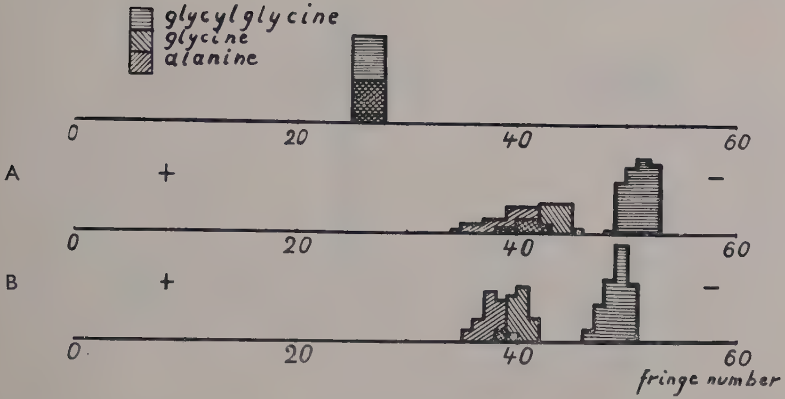

Fig. 2. Electrophoresis of 0.1 % glycine, 0.1 % alanine and 0.2 % glycylglycine in formate buffer, pH 2.61. Velocity of the liquid flow: 0.28 ml. per hour per fringe. The diagram shows the weight concentrations of the substances in the injected solution (first line) and the filtrates (second and third lines) plotted against the fringe number. A. Ionic strength of the buffer: 0.1 Electric current: 66 mA. Voltage drop over the apparatus: 1040 volt. B. Ionic strength of the buffer: 0.2. Electric current: 140 mA. Voltage drop over the apparatus: 940 volt.

ments with the amino acids, giving a velocity of flow of only 0.15 ml per hour per fringe, whereas 0.28 ml per hour per fringe was obtained in the amino acid experiments.

**Results.** All accurate mobility data in the literature refer to the migration in free liquid. Recently it has been shown by KUNKEL and TISELIUS (7) that the mobilities of the serum proteins are hardly lower in filter paper systems than in free solution. But the effective path of migration is greater in the former case than in the latter, and varies with the kind of paper used. The apparent displacements, measured relative to the apparatus, are therefore diminished when the migration takes place in a paper system. In the absence of apparent mobility data referring to the migration in the paper, the resolving power of the apparatus is here related to mobilities valid in free solution. If specific influences of the filter paper system upon the ions are not pronounced, such data are useful in viewing the possibilities of the method and in choosing proper separation conditions in new experiments.

By relating the zone displacements at the level of outflow to the mobility differences between the zones, a mobility scale is obtained. Each fringe corresponds to 6 mm of the length of the paper. If the zone distances are measured in the two last experiments of Fig. 2 and Fig. 3, where the zones are best defined, the figures in the first line of Table 1 are obtained. These displacements should be related to the mobility differences of the substances forming the bands. The mobilities of glycine, alanine and glycylglycine have been determined by SVENSSON, BENJAMINSSON and BRATTSTEN (12). These mobilities are valid in formic acid solution at the ionic strength 0.01. They allow the calculation of the mobilities in the formate buffer of ionic strength 0.2, if the concentration dependence of the mobilities upon the free acid and the ionic strength is known. These functions have not been determined for the amino acids, however. The recalculation performed here is instead founded on the results of an investigation

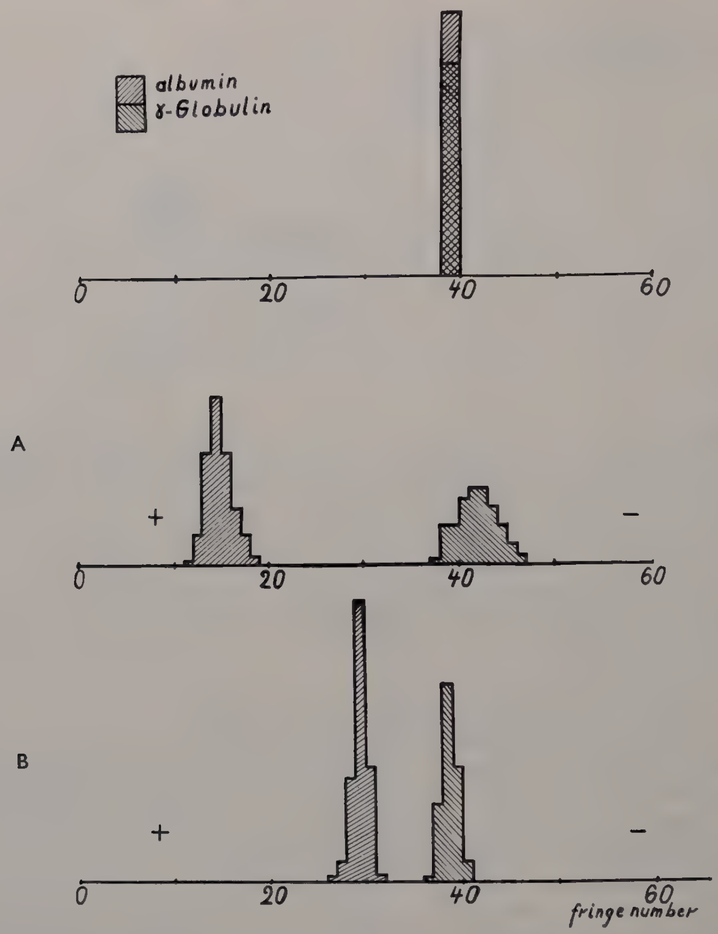

Fig. 3. Electrophoresis of 0.7 % serum albumin and 0.56 % $\gamma$-globulin in phosphate buffer of pH 7.70. Velocity of the liquid flow: 0.15 ml. per hour per fringe. The diagram shows the weight concentrations of the substances in the injected solution (first line) and the filtrates (second and third lines) plotted against the fringe number. A. Ionic strength of the buffer: 0.05. Electric current: 32 mA. Voltage drop over the apparatus: 1000 volt. B. Ionic strength of the buffer: 0.2. Electric current: 55 mA. Voltage drop over the apparatus: 470 volt.

where the mobilities of a number of substances of the acetic acid type have been determined as functions of the medium (I. B., unpublished).

The following values are used:

| | alanine | glycine | glycyl-glycine |
|---|---|---|---|
| u.$10^5$ | 3.9 | 4.7 | 7.3 |

They give the mobility difference $3.4 \times 10^{-5}$ for glycylglycine and alanine and $2.6 \times 10^{-5}$ for glycylglycine and glycine. The mobilities of the serum components

*Table 1.*

The Resolution of Representative Mixtures of Amino Acids and Proteins (in mobility units per cm at the level of outflow).

1. 0.1 % glycine
0.1 % alanine
0.2 % glycylglycine
in formate buffer,
$\mu=0.2$, pH=2.61
I=140 mA
vel. of flow: 0.28 ml
per hour per fringe

2. 0.7 % albumin
0.56 % γ-globulin
in phosphate buffer
$\mu=0.2$, pH=7.70
I=55 mA
vel. of flow: 0.15 ml
per hour per fringe

| | 1 | | 2 |
|---|---|---|---|
| | glycylglycine -alanine | glycylglycine -glycine | albumin -γ-globulin |
| band distance (cm) | 6.8 | 5.1 | 5.4 |
| $\Delta u \times 10^5$ . . . . . . . . . | 3.4 | 2.6 | 4.4 |
| $u \times 10^5$ per cm. . . . | 0.50 | 0.51 | 0.815 |

in moving boundary electrophoresis are well known quantities; thus for serum albumin in phosphate buffer of ionic strength 0.2 and pH 7.68 the mobility is $-5.0 \times 10^{-5}$ and for γ-globulin under the same conditions $-0.6 \times 10^{-5}$. The mobility difference between these proteins, $4.4 \times 10^{-5}$, together with those of the amino acids are put in the second line of Table 1. The third line gives the mobility equivalents of unit distance at the level of outflow for the two electrophoretic systems. This resolution, $(0.5–0.8) \times 10^{-5}$ mobility units per cm, will prove sufficient for many purposes. It should be emphasized that these results are obtained under conditions where boundary anomalies are depressed and a minimum of dilution occurs. With the same current density and velocity of flow, the separation can be increased either by decreasing the salt concentration in the system or by reducing both the protein (amino acid) and the buffer salt concentrations. In the former case the increased separation is gained, more or less, at the cost of the sharpness of the zones. In both instances, therefore, the increased resolution is parallelled by increased dilution of the separated substances. Such conditions nevertheless might be advantageous, if the separation process can be followed by some suitable concentration procedure, for instance concentration at a conductivity gradient in a repeated run of the dilute fractions. To obtain the highest possible yield, the bands should of course be as broad as is compatible with full resolution of the components.

The two types of experiments reported here illustrate the fact that electrophoretic separation of amino acids is generally more difficult to perform than separation of proteins, especially if the amino acids belong to the same group. These difficulites arise from dissimilarities in the electrochemical properties of amino acids when compared with proteins. The specific charge of an amino acid is much lower than that of a protein. At a certain ionic strength a greater concentration of a protein (per weight) can therefore be used than of an amino acid without conductivity disturbances. Furthermore, most proteins have well defined isoelectric points, whereas the isoelectric state of the amino acids prevail over several pH units in the neutral range. The ionic forms with high mobilities, which are of course best suited for electrophoretic separations, are often found only in rather acid and alkaline media. Both the amino acids and the peptide used here belong to the neutral group. In the acid dissociation range, the mobilities are most favourable for separation at pH 2.6 (see Fig. 5, ref. 13), but at this pH the rather high concentration of the hydrogen ion involves certain disadvantages. In the rear boundaries, fast hydrogen ions are to a relatively great extent substituted for the amino acid cations. In the buffered solutions used here, the effect of this exchange on the conductivity is probably more important than the pH effect, resulting in a blurring of these boundaries. The trailing of the amino acid boundaries in Fig. 2, A, may to some extent be due to this effect. In addition, the contribution of the hydrogen ions to the conductivity of the formate buffer was considerable. The conductivity of this buffer was $8.81 \times 10^{-3}$, whereas that of the phosphate buffer of the same ionic strength, at pH 7.70, was $5.51 \times 10^{-3}$. A correspondingly higher current is therefore needed in the formate system than in the phosphate system to maintain the same field strength. For these reasons it is evident that the separation of glycine and alanine in the acid medium is more difficult than the separation of two serum globulin components in the neutral range, although the mobility differences are of the same order of magnitude. For separations in the high alkaline range similar considerations apply, but the effects are generally less pronounced in such cases.

With regard to the possibilities of increasing the absolute separation efficiency of the apparatus, the experiments suggest that some improvements can be made. In the highly conducting formate system, 133 watts were applied without difficulties in eliminating the generated heat. This demonstrates that the cooling system is operatively efficient. In the phosphate system of ionic strength 0.2, only 26 watts could be used, however. In this case the efficiency of the neutralization of the electrode reaction products limits the electrical capacity, and a reconstruction of the electrode compartments to allow a faster transfer of the electrode liquids is needed. The capacity of the apparatus also can be enhanced by increasing the thickness of the filter paper column. Simultaneously, the constancy of the cross sectional area should be adjusted. This can be done by the insertion of thin lucite lamellas of proper height in cuts of the paper along the edges. In the present apparatus, the pressure from the brass frames is exerted on the paper, and a careful adjustment is necessary to avoid uneven pressure, which causes disturbances in the course of the liquid flow. This difficulty will disappear if the pressure of the frames is exerted instead upon lamellae of equal height.

As already pointed out, this investigation is restricted to questions concerning the absolute separation conditions in an apparatus of this type. To serve the purpose of performing a continuous separation, however, the apparatus should also be developed and tested with regard to the time constancy of the processes, especially the liquid flow. In order to increase the precision of this process in long period runs, it is desireable that the rather frail paper fringes, which are now in use to supply the liquid, are exchanged for some adjustable, automatically working device. With these improvements, the apparatus can be expected to work well, and the method suggests itself to many problems, where the capacity of the ordinary filter paper electrophoresis is too low, although the available quantities of solution to be separated are too small for the glass powder apparatus.

## SUMMARY

An apparatus for small scale fractionation according to the method of continuous zone electrophoresis is described. The separation takes place in sheets of filter paper clamped between glass plates. Circulating water is used to cool the apparatus. The resolution obtainable with this arrangement has been tested in separation experiments with substances of known mobilities. A mixture of glycine, alanine and glycylglycine in acid medium and a mixture of serum albumin and γ-globulin in neutral medium were used for this purpose. These experiments were also arranged to give some information of the most suitable separation conditions in various cases, and to demonstrate some dissimilarities between amino acids and proteins in electrophoresis.

## Acknowledgements

The authors are much indebted to Professor A. Tiselius for his kind interest in this work and for laboratory facilities put at our disposal.

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REFERENCES. 1. **Janssen, L. W.**: unpublished. — 2. **Svensson, H.** and **Brattsten, I.**: Arkiv Kemi *1* (1950) 401. — 3. **Grassman, W.** and **Hannig, K.**: Z. angew. Chem. *62* (1950) 170. — 4. ——: Naturwiss. *37* (1950) 397. — 5. **Cremer, H. D.** and **Tiselius, A.**: Biochem. Z. *320* (1950) 273. — 6. **Wieland, Th,** and **Fischer, E.**: Naturwiss. *35* (1948) 29. — 7. **Durrum, E. L.**: J. Am. Chem. Soc. *72* (1950) 2943. — 8. **Turba, F.** and **Enenkel, H. J.**: Naturwiss. *37* (1950) 93. — 9. **Kunkel, H.** and **Tiselius, A.**: in press. — 10. **Svensson, H.**, **Benjaminsson, A.** and **Brattsten, I.**: Acta Chem. Scand. *3* (1949) 307.

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# Evidence for complex formation between peptides and trichloracetic acid[1]

By I. GORDON FELS

With 2 figures in the text

In the course of study of large molecular weight peptides from diverse sources, it became apparent that their electrophoretic patterns were remarkably similar (Fig. 1). Although this in itself is not singular, the observation that all cases possessed a characteristic and stable boundary with a negative refractive index gradient (henceforth referred to as the inverted boundary in this paper), warranted further study. This finding is in contrast to the blurring effects previously obtained with peptide mixtures (1, 2). The present communication presents evidence for the formation of complexes between peptides and trichloracetic acid, and methods by which they may be eliminated.

## Experimental

The peptides were obtained from Witte peptone, [Rostock], (3), calf's liver (3), and unripe peas.[2] In the case of peptone and liver, the original procedure was modified slightly to give a cleaner looking product. In each instance, the final step consisted of precipitation of the concentrated peptide solution with 10 % trichloracetic acid (final volume), multiple extraction with ether, redissolution of the peptides in water, and freeze drying with alcohol and solid $CO_2$. Mobility measurements were made in the improved Tiselius apparatus using the inclined slit optical system (4). However, because preliminary dialysis could not be used, the direct use of the material in the electrophoresis experiments resulted in enlarged false resting boundaries, due to conductivity differences between the buffer and solution. In spite of this, no discernible convections were present, and the measurements in general were reproducible. The sharp descending boundaries were particularly well suited for these determinations, hence all measurements were restricted to the descending limb. All experiments were carried out in sodium diethylbarbiturate (veronal)-HCl buffer of ionic strength 0.1 and pH 7.92, and lasted about an hour, during which time the inverted boundary

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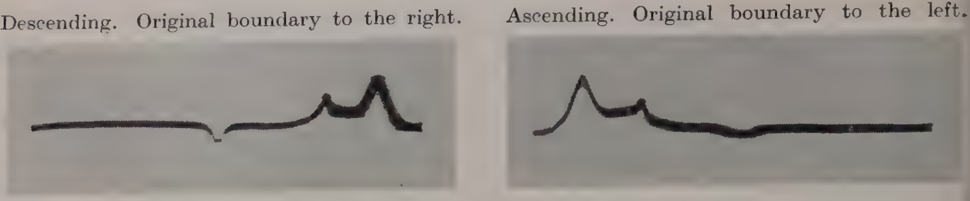
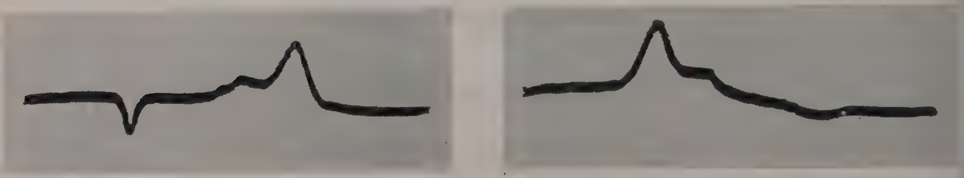
[1] The joint sponsorship of the American Cancer Society and the American Scandinavian Foundation is gratefully acknowledged.

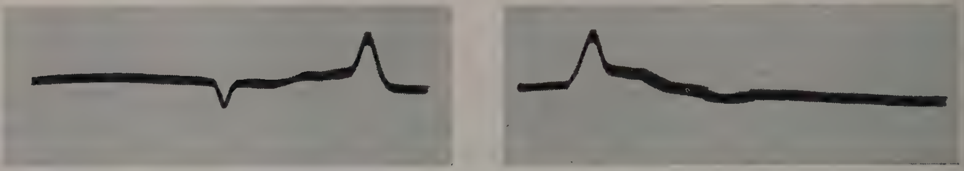
[2] The peptides from peas were kindly provided by I. D. RAACKE-FELS. They were obtained by a method similar for that of liver.



a. Peptides from Witte peptone after 1860 seconds.



b. Peptides from calf's liver after 1860 seconds.



c. Peptides from unripe peas after 1860 seconds.

Fig. 1.

migrated about 2.5 cm. The temperature used in the experiments was 0.6° ± 0.1°. For calculating the field strength, the conductivity of the bottom cell was used. All mobilities are an average of seven values calculated from one experiment. In view of the experimental conditions and the number of experiments performed the mobility calculations are not regarded as final. The pertinent data are given in Table I, from which it may be seen that the mobility of the inverted boundary from all sources is identical.

In view of the fact that all preparations included a step involving trichloracetic precipitation, it was at first believed that the precipitant had been occluded in the precipitation, giving rise to the inverted boundary in all cases. Evidence in this direction was supplied by the observation that the inverted boundary did not appear in the pattern if the trichloracetic acid step was omitted in the precipitation. Furthermore, electrophoresis of trichloracetic acid itself in veronal buffer yielded an inverted boundary of approximately the same mobility (Table I). On the other hand, it was impossible to eliminate the inverted boundary by extraction with ether. After two hours' extraction in the Soxlet, the peptides were dissolved in water, and the solution repeatedly extracted with ether. The percentage area of the inverted boundary in the extracted material, as determined with a planimeter, was identical with that of unextracted peptides, i.e., 14 % for a concentration of 0.4 % peptides. Isolation of material behind the inverted boundary (ascending limb) yielded a small amount of substance which reacted positively with ninhydrin. Inasmuch as the veronal interfered with the ninhydrin reaction, it was first necessary to reprecipitate the

*Table I*

Mobility data for inverted boundary.

| Source | Percent concentration | Conductivity of solution × $10^{-3}$ ohm cm | Average field strength[1] volt/cm | Average velocity × $10^5$ cm/sec | Average mobility × $10^5$ $cm^2$/volt sec |
|---|---|---|---|---|---|
| Witte peptone..... | 1.0 | 3.28 | 7.45 | 102.1 | —13.7 |
| Liver............. | 0.6 | 3.29 | 6.96 | 96.7 | —13.9 |
| Pea seeds......... | 0.6 | 3.37 | 5.84 | 81.2 | —13.9 |
| Trichloracetic acid | ca. 0.1 | 3.15 | 7.21 | 101.7 | —14.1[2] |

peptides with trichloracetic acid, and wash the precipitate with ether. Paper chromatograhpy of the isolated material, using *n*-propanol, *n*-butanol, 0.1 *N* HCl (2:1:1) as solvent, resulted in two spots, only one of which was mobile.

This evidence points to the possibility that the trichloracetic acid might be bound to the peptides in the form of a complex.[3] The binding of trichloracetic acid by proteins has been reported (5, 6). Similarly, definite salts between trichloracetic acid and amino acids have been prepared (7). Interestingly, these amino acid trichloracetates cannot be decomposed with benzene, whereas with acetone, all the trichloracetic acid can be extracted from them (7).

With this in mind, the solid peptides obtained from Witte peptone were extracted three times with 50 ml acetone. The electrophoretic pattern of the extracted material did not contain the inverted boundary. This similarity of behavior suggests that the peptide complexes are salt-like in nature, analogous to the amino acid trichloracetates, and that they are capable of dissociation in polar solvents. The inability of ether to decompose the complexes in aqueous solution might be accounted for by the greater solubility of trichloracetic acid in water, although this remains to be established. One would anticipate further that during the electrophoresis of a trichloracetate complex, the migration of the trichloracetate ion should be observable under conditions of appreciable dissociation. Observations of this type have been made with protein-alkyl sulfate complexes (8). For this purpose, glycine trichloracetate was prepared by the method of FITZGERALD (7). It had a melting point of 133° (corrected), exactly as that recorded. Although this amino acid salt cannot be considered a complex in the usual sense of the term, nevertheless it was hoped that its electrophoretic behavior would contribute to understanding the peptide complexes. Here again considerable difficulties were encountered as in the case of trichloracetic acid, and concentrations of about 0.1 % were the only ones that could be used. The electrophoretic pattern of glycine trichloracetate is shown in Fig. 2. It is practically identical to that obtained with trichloracetic acid (not shown). No mobility measurements are given because of convection disturbances in all experiments. Most of the values obtained, however, approximate these in previous experiments.

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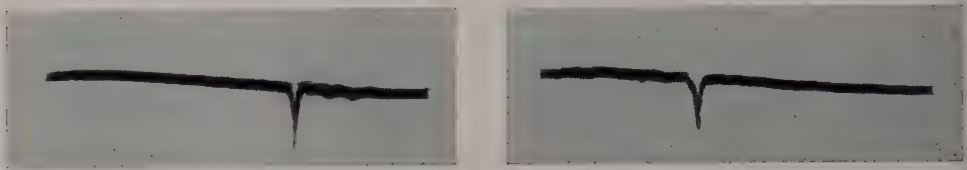
[1] Inasmuch as the current during an experiment is averaged, the field strength is likewise an average value.

[2] Considerable difficulties were met due to appreciable pH and conductivity changes in the presence of the strong acid. The resulting convections made measurements impossible. Solutions greater than 0.1 % inevitably crystallized out when equilibrated at the low temperature, whereas more dilute solutions gave too small a boundary to be followed accurately. The above value therefore, is an average of measurements from two experiments, taken before convections became evident.

[3] The common usage of the term "complex" is unfortunate, inasmuch as it presupposes a relationship to the coordination compounds. The term as used in this paper will refer to compounds formed by the stoichiometric binding of ions irrespective of the type of binding.

Descending. Original boundary to the right. Ascending. Original boundary to the left.



Glycine trichloracetate after 600 seconds. The irregular base line is due to convection disturbances.

Fig. 2.

From the fact that peptides can be isolated behind the ascending inverted boundary, when they are used in the electrophoresis, one is led to believe that the dissociation of the complexes is not complete. This infers that the complexes are capable of formation at a pH where the components possess the same charge, an observation which has ample verification in the literature (9–11).

It can readily be shown that the inverted bound is not a false moving boundary predictable from a buffer system containing two negative ions (4). The use of veronal without HCl (pH 9.87) was found to have no effect upon the electrophoretic pattern of the peptides.

The boundary inversion is not in itself a novelty (12, 13), but represents a negative refractive index gradient.[1] It is not present in borate at the same pH, in phosphate at 7.08, or acetate at 4.25. The ions of these buffers probably are also too fast in comparison to trichloracetate to give stable boundaries. The mobility of veronal ion is very close to that of trichloracetate (14), resulting in a sharp measurable boundary when pH or conductivity effects are absent.

Of the other boundaries in Fig. 1 a b c, the middle peak blurs rapidly, making measurement impossible. The large boundary is stationary, and probably is a false resting boundary analogous to those found in protein systems. It is interesting to note that the stationary boundary becomes greatly diminished when the inverted boundary is removed, suggesting that its large size is due to the high specific charge of the peptide complexes as well as pH differences at the boundary. At the same time, the rising boundaries become sharp, whereas those in the descending limb become diffuse.

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[1] This is indicated from measurements of the refractive index of veronal and veronal plus trichloracetate solutions whose concentrations have been adjusted so as to similate the conditions during an electrophoresis experiment. Solutions containing trichloracetate and veronal are found to have lower indices than that of verona alone. Thus for example a solution containing 100 mM veronal and 12 mM trichloracetic acid had a refractive index of 1.3358 whereas a solution containing 112 mM veronal had an index of 1.3362 at 7° C.

In other experiments, the removal of the inverted boundary also was found possible by boiling the peptide solution for 15 minutes. This is due presumably to the destruction of the trichloracetic acid, shifting the equilibrium until all the acid is consumed. The destruction of trichloracetic acid by boiling has been used for a long time in protein chemistry (15). In view of the routine use of trichloracetic acid in obtaining large peptides, it is recommended that the acid be eliminated by extraction with a polar solvent, such as acetone, in which the peptides are insoluble. In certain cases, losses are unavoidable. The alternate procedure is to boil the solution for 15 minutes.

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BIBLIOGRAPHY. 1. **M. Annetts,** Biochem. J. *30,* 1807, 1936. — 2. **A. Tiselius** and **I. B. Eriksson-Quensel,** Ibid. *33,* 1752, 1939. — 3. **H. Borsook,** et al., J. Biol. Chem. *179,* 705, 1949. — 4. **H. Svensson,** Ark. f. Kemi, Mineral. Geol. *22A,* No. 10, 1946. — 5. **R. K. Schofield** and **L. W. Samuel,** Nature *134,* 665, 1934. — 6. **J. Steinhardt, D. H. Fugitt,** and **M. Harris,** J. Res. Nat. Bureau Standards, *26,* 293, 1941. — 7. **M. E. Fitzgerald,** Roy. Soc. Canada *31,* 153, 1937. — 8. **F. W. Putnam** and **H. Neurath,** J. Biol. Chem. *159,* 195, 1945. — 9. **H. P. Lundgren, D. W. Elam, R. A. O'Connell,** Ibid. *149,* 183, 1943. — 10. **M. Harris,** J. Res. Nat. Bureau Standards *10,* 475, 1933. — 11. **I. M. Klotz** and **M. Urquhart,** J. Physical and Colloid Chem. *53,* 100, 1949. — 12. **I. Brattsten** and **H. Svensson,** Act. Chem. Scand. *3,* 359, 1949. — 13. **H. Svensson, A. Benjaminsson,** and **I. Brattsten,** Act. Chem. Scand. *3,* 307, 1949. — 14. **L. G. Longsworth,** J. Physical and Colloid Chem. *51,* 171, 1947.

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# Über einige Beziehungen betreffend die Ionisation mehrbasischer Säuren

Von Erik Söderbäck

Im Jahre 1934 habe ich eine rationelle Theorie der Berechnung von Dissoziationskonstanten für ein-, zwei- und dreibasische Säuren veröffentlicht.

Es wurden in Zusammenhang damit gewisse allgemeine Sätze angeführt, die für Säuren beliebiger Basicität gelten. In einigen Fällen wurden diese Sätze ohne Beweis mitgeteilt. In der jetzigen Abhandlung möchte ich eine Komplettierung in dieser Hinsicht erstatten sowie einige neue allgemeine Beziehungen hinzufügen.

Die Bezeichnungen der in den Formeln auftretenden Grössen sind mit einigen Ausnahmen dieselben wie in meiner früheren Abhandlung. Da diese im folgenden öfters zitiert werden muss, wird sie schlechthin mit I bezeichnet.

Es bedeuten

| | |
|---|---|
| $c$ | molare Konzentration von Säure nebst deren Alkalisalz |
| $h$ | molare Konzentration des Wasserstoffions |
| $oh$ | molare Konzentration des Hydroxylions |
| $a_H$ | Wasserstoffionenaktivität |
| $K'$ | klassische oder Konzentrationsdissoziationskonstante, Indices in Bezug auf Dissosiationsstufe |
| $K$ | unvollständige Dissoziationskonstante, berechnet aus der Wasserstoffionenaktivität und molaren Konzentrationen der übrigen am Gleichgewicht teilnehmenden Stoffe |
| $q_p$ | $K_p/K_{p+1}$; $H = K/a_H = K'/h$ |
| $\alpha$ | Dissoziationsgrade der Säure Indices in Bezug auf Valenz des Säureanions |
| $\alpha_0$ | Dissoziationsrest |
| $\Gamma$ | Ionale Konzentration der Säurelösung also der doppelte Betrag der Ionenstärke |
| $P(K_m)$ | Produkt von $K$ mit Indices von 1 bis $m$ |
| $P(q_m)$ | Produkt von $q$ mit Indices von 1 bis $m$ |

Es sei eine $n$-basische ungeladene Säure $H_nA$ in wässriger Lösung gegeben; diese Lösung sei eventuell durch die leichtlösliche starke Base MeOH teilweise oder ganz neutralisiert. Wir setzen voraus, dass die Me-Salze der Säure sämtlich löslich und bei den angewandten Verdünnungen vollständig in Metallion und Säureion dissoziiert sind.

Die Bedingung der Elektroneutralität der Lösung gibt die folgende Gleichung

$$[\mathrm{Me}] + h - oh = c\,(a_1 + 2\,a_2 + \cdots n\,a_n)\,. \tag{1}$$

Ferner gilt

$$a_0 + a_1 + \cdots a_n = 1 \tag{2}$$

Wir machen nun den Ansatz

$$\frac{[\mathrm{Me}] + h - oh}{c} = B \tag{3}$$

wodurch (1) folgendermassen geschrieben werden kann

$$B = a_1 + 2\,a_2 + \cdots n\,a_n\,. \tag{4}$$

Wenn die Veränderung, welche die Säure bei fortschreitender Neutralisation erleidet, darin besteht, dass undissoziierte Säure in primäres Anion, und ein solches von niedriger Valenz in eines von höherer verwandelt werden, muss also nach (4) bei fortschreitender Neutralisation $B$ wachsen. Sein Maximalwert wird also erreicht, wenn die Säure ausschliesslich als Anion der Valenz $n$ existiert und ist somit *der Basicität $n$ gleich.* Wenn diese unbekannt ist, kann sie also durch Feststellen, welcher ganzen Zahl sich $B$ bei fortschreitender Neutralisation der Säure nähert, ermittelt werden.

Die physikalische Bedeutung der Quantität $B$ geht aus (4) hervor, $B$ stellt die durchschnittliche Ladung des Säuremoleküls dar.

Bei Aufnahmen von Titrationskurven werden diese gewöhnlich durch die $n$ Äquivalenzpunkte in $n$ Gebiete zerlegt. Ich habe in I die Grenzpunkte der Gebiete nach ganzzahligen Werten von $B$ bestimmt, und dieser Einteilungsgrund wird hier beibehalten. Der Name Neutralisationsgebiet wird zwar bei dieser Aufteilung nicht immer adäquat, da es bei sehr starken Säuren eintreffen kann, dass die unneutralisierte Säure einen $B$-Wert grösser als 1 hat.

Was die Berechnung von $B$ betrifft, sei auf I s. 5–6 verwiesen.

Das Massenwirkungsgesetz auf die $n$ Dissoziationsstufen der Säure bezogen, führt zu den folgenden $n$ Gleichungen

$$\frac{a_1}{a_0} = \frac{K_1}{a_H};\ \frac{a_2}{a_1} = \frac{K_2}{a_H} \cdots \frac{a_n}{a_{n-1}} = \frac{K_n}{a_H}\,.$$

Multiplikation von diesen gibt

$$a_1 = a_0\,\frac{K_1}{a_H};\ a_2 = a_0\,\frac{K_1 K_2}{a_H^2};\ \ldots a_n = a_0\,\frac{P(K_n)}{a_H^n}\,. \tag{5}$$

Ferner wird durch Multiplikation von (2) mit $B$ und Subtraktion von $B$ davon erhalten

$$B\,a_0 + (B-1)\,a_1 + \cdots (B-n)\,a_n = 0\,. \tag{6}$$

Einsetzen in dieser Gleichung von den $\alpha$-Werten gemäss (5) liefert

$$B + (B-1)\frac{K_1}{a_H} + \cdots (B-n)\frac{P(K_n)}{a_H^n} = 0\,^{1} \tag{7}$$

oder, wenn $H$ für $K/a_H$ eingeführt wird

$$B + (B-1)\,H_1 + \cdots (B-n)\,P(H_n) = 0. \tag{7 a}$$

Bezeichnen wir die Terme des linken Membrums von (7 a) mit $t_0$, $t_1$ u. s. w., folgt sofort

$$t_0 + t_1 + \cdots t_v \geq 0 \quad (v \leq n).$$

Aber dann muss auch gelten

$$p_0 t_0 + p_1 t_1 + \cdots p_v t_v \geq 0 \tag{8 a}$$

$$(p > 0;\ p_0 \geq p_1 \geq p_2 \text{ u. s. w.})$$

und zwar weil diese Summe immer in Partialsummen von konsekutiven Termen mit gleichen Koeffizienten, wo der erste Term immer $t_0$ enthält, zerlegt werden kann. Ferner gilt

$$t_n + t_{n-1} + \cdots t_u \leq 0 \quad (u \leq n)$$

und also auch

$$s_n t_n + s_{n-1} t_{n-1} + \cdots s_u t_u \leq 0 \quad (u \leq n) \tag{8 b}$$

$$(s > 0;\ s_n \geq s_{n-1} \geq s_{n-2} \cdots)$$

In Gleichung (7 a) können durch die Quotienten $q$ alle $H$ in ein einziges als unabhängige Variable ausgedrückt werden. Wählen wir als diese $H_n$, nimmt (7 a) die Form

$$B + (B-1)\,P(q_{n-1})\,H_n + (B-2)\frac{P(q_{n-1})^2}{q_1}H_n^2 + \\ + (B-3)\frac{P(q_{n-1})^3}{q_1^2 q_2}H_n^3 + \cdots = 0. \tag{9}$$

Aus elementaren Sätzen der Theorie algebraischer Gleichungen geht hervor, dass diese Gleichung *eine* aber nur *eine* positive Wurzel hat, das letzte weil die Koeffizienten für gegebenes $B$ nur *einen* Zeichenwechsel erleiden. Es ist also möglich beim Auflösen der Gleichung $H_n$ und damit auch die übrigen $H$ eindeutig zu bestimmen.

Aus Gleichung (7 a) folgt, dass, wenn bei konstantem $B$ eines oder mehrere von den $H$ wachsen dürfen, eines oder mehrere von den übrigen $H$ abnehmen

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[1] Diese Gleichung ist zuerst von AUERBACH und SMOLCZYK obgleich in etwas anderer Form für $n = 2$ und 3 abgeleitet worden und später von BJERRUM und UNMACK bei der Berechnung von Dissoziationskonstanten für Zitronensäure benutzt worden.

müssen. Bezeichnen wir das linke Membrum von (7 a) mit $M$ und bilden die partielle Ableitung $\frac{\partial M}{\partial H_p}$, so ergibt sich

$$\frac{\partial M}{\partial H_p} = \frac{P(H_p)(B-p) + P(H_{p+1})(B-p-1) + \cdots P(H_n)(B-n)}{H_p}.$$

Nach Ungleichung (8 b) ist aber $\frac{\partial M}{\partial H_p}$ negativ, weshalb das totale Differential

$$dM = \frac{\partial M}{\partial H_1} dH_1 + \cdots \frac{\partial M}{\partial H_m} dH_m$$

wenn alle $H$ wachsen, negativ wird, und die Gleichung (7 a) deshalb nicht bestehen kann. Eines oder mehrere von den $H$ können also nicht wachsen, ohne dass eines oder mehrere der übrigen abnehmen.

Denken wir uns nun die $H$ als Funktionen von $B$ und den $q$ durch Gleichung (9) bestimmt. Dann können wir sofort das Zeichen der Ableitungen $\frac{\partial H_p}{\partial q_r}$ bestimmen. Weil $q_r = \frac{H_r}{H_{r+1}}$, werden $H_r$ und alle $H$ mit Indices kleiner als $r$ bei anwachsendem $q_r$ steigen, $H_{r+1}$ aber, und alle $H$ mit Indices grösser als $r+1$ sinken. Es gilt also die Regel $\frac{\partial H_p}{\partial q_r}$ ist positiv, wenn $p \leq r$ ist, sonst negativ.

Wenn die $n-1$ $q$ sämtlich sehr gross sind, hat also $H_n$ für einen fixierten $B$-Wert seinen *kleinsten*, $H_1$ aber seinen *grössten* Betrag.

## Dissoziationsgrade

Eine Frage von Bedeutung für die Berechnung vom Zustand in Säurelösungen ist, *wie* die Dissoziationsgrade und der Dissoziationsrest verändert werden, wenn die Quotienten $q$ bei konstantem $B$ wachsen.

Namentlich interessiert uns das Zeichen der partiellen Ableitungen $\frac{\partial \alpha}{\partial q}$.

Es wurde oben bewiesen, dass wenn ein Quotient, sei es $q_r$, anwächst, die übrigen aber konstant bleiben, die $H$ mit Indices gleich $r$ oder kleiner wachsen, die mit Indices gleich $r+1$ oder grösser abnehmen. Nun gilt $H_p = \frac{\alpha_p}{\alpha_{p-1}}$. Logarithmieren und Differenziieren gibt

$$\frac{\Delta H_p}{H_p} = \frac{\Delta \alpha_p}{\alpha_p} - \frac{\Delta \alpha_{p-1}}{\alpha_{p-1}}. \tag{10}$$

Für alle $H$ mit $p \leq r$ ist also das rechte Membrum von (10) positiv, für $p > r$ hingegen negativ.

Wir wollen nun zunächst untersuchen, ob $\Delta \alpha_0$ bei wachsendem $q_r$ positiv sein kann. Wenn dies der Fall ist, muss offenbar gemäss (10) auch $\Delta \alpha_1$, $\Delta \alpha_2$

u. s. w. wenigstens bis zu $\Delta \alpha_r$ positiv sein. Allmählich muss aber ein Übergang zu negativen $\Delta \alpha$ eintreffen, und wenn dies einmal geschehen ist, müssen offenbar alle nachfolgende auch negativ sein wegen des negativen Vorzeichens von $\Delta H_p$. Die Reihe der $\Delta \alpha$ würde also, wenn man mit $\Delta \alpha_0$ beginnt, in zwei Gruppen zerfallen, zunächst eine positive und dann eine negative und wir supponieren, dass der letzte positive Term $\Delta \alpha_u$ ist. Differentiierung von (2) und (4) gibt

$$\Delta \alpha_0 + \Delta \alpha_1 + \cdots \Delta \alpha_n = 0 \tag{11 a}$$

$$\Delta \alpha_1 + 2 \Delta \alpha_2 + \cdots n \Delta \alpha_n = 0. \tag{11 b}$$

Diese beiden Gleichungen können aber nicht gleichzeitig bestehen, wenn $\Delta \alpha_0$ bis zu $\Delta \alpha_u$ positiv sind. Es gilt nämlich

$$\sum_1^u p \Delta \alpha_p < u \sum_1^u \Delta \alpha_p$$

und ferner

$$\sum_{u+1}^n p \Delta \alpha_p < (u+1) \sum_{u+1}^n \Delta \alpha_p$$

und somit

$$u \sum_1^u \Delta \alpha_p + (u+1) \sum_{u+1}^n \Delta \alpha_p > \sum_1^n p \Delta \alpha_p.$$

Da das letzte Glied dieser letzten Ungleichung gleich 0 ist, muss also das linke Membrum positiv sein. Nun ist aber für $\Delta \alpha_0 > 0$

$$\Delta \alpha_1 + \Delta \alpha_2 + \cdots \Delta \alpha_n < 0$$

und folglich

$$u \sum_1^u \Delta \alpha_p + (u+1) \sum_{u+1}^n \Delta \alpha_p$$

a fortiori negativ.

Der Ansatz $\Delta \alpha_0 > 0$ führt somit, wenn $q_r$ anwächst, zu einer Absurdität und $\Delta \alpha_0$ muss also negativ sein. Allmählich muss aber ein Übergang zu positiven $\Delta \alpha$ eintreffen, da die Summe der $\Delta \alpha$-Reihe ja Null ist. *Wann* der Zeichenwechsel einsetzt, lässt sich nicht entscheiden, es kann aber behauptet werden, dass $\Delta \alpha_r$ positiv ist. Denn, wenn dies nicht der Fall sei, dann müsste gemäss (10) auch $\Delta \alpha_{r+1}$ und alle folgende Glieder der Reihe negativ sein, was zu einer offenbaren Absurdität führt.

Es lässt sich auch beweisen, dass $\Delta \alpha_n$ negativ sein muss. Denn, wenn $\Delta \alpha_n$ positiv wäre, dann würde auch laut (10) $\Delta \alpha_{n-1}$ es sein und alle vorhergehende $\Delta \alpha$ wenigstens bis zu $\Delta \alpha_r$. Die Reihe der $\Delta \alpha$ würde somit in zwei Gruppen zerfallen, zunächst eine negative mit $\Delta \alpha_0$ beginnend, deren letztes Glied $\Delta \alpha_v$ sei, dann eine positive. Es würden dann die Ungleichungen bestehen

$$v \sum_1^v \Delta \alpha_p < \sum_1^v p \Delta \alpha_p; \quad (v+1) \sum_{v+1}^n \Delta \alpha_p < \sum_{v+1}^n p \Delta \alpha_p$$

und somit

$$v \sum_1^v \Delta \alpha_p + (v+1) \sum_{v+1}^n \Delta \alpha_p < \sum_1^n p \Delta \alpha_p.$$

Da nun

$$\sum_1^n p \varDelta a_p = 0$$

ist, würde also die Ungleichung bestehen

$$v \sum_1^v \varDelta a_p + (v+1) \sum_{v+1}^n \varDelta a_p < 0,$$

was aber offenbar absurd ist da

$$\sum_1^n \varDelta a_p > 0 \quad \text{für} \quad \varDelta a_0 < 0.$$

Der Ansatz $\varDelta a_n > 0$ führt somit zu einer neuen Absurdität, und $\varDelta a_n$ muss also negativ sein.

Das Resultat der Untersuchung ist demnach, dass die Reihe der $\varDelta a$ in drei Gruppen zerlegt werden muss, wenn $q_r$ anwächst, zuerst eine negative, dann eine positive und dann wieder eine negative. Für eine zweibasische Säure beschränkt sich die Zahl der Glieder jeder Gruppe zu einem einzigen.

Es gilt also allgemein

$$\frac{\varDelta a_0}{\varDelta q_r} < 0; \quad \frac{\varDelta a_n}{\varDelta q_r} < 0; \quad \frac{\varDelta a_r}{\varDelta q_r} > 0$$

oder beim Übergang zu Limes

$$\frac{\partial a_0}{\partial q_r} < 0; \quad \frac{\partial a_n}{\partial q_r} < 0; \quad \frac{\partial a_r}{\partial q_r} > 0 \quad (0 < r < n).$$

Die physikalische Bedeutung der zwei ersten Ungleichungen ist die folgende. Wir denken uns eine Reihe von Säuren derselben Basicität zu demselben $B$-Wert neutralisiert. Für eine Säure, deren sämtliche Quotienten $q$ grösser sind als diejenigen einer anderen, sind über die ganzen $B$-Skala die Bruchteile der Säure die undissoziiert und als Anion der Valenz $n$ in der Lösung vorkommen, beide kleiner als in der anderen, wo die Quotienten kleiner sind.

Besonders einfach werden die Formeln für die Dissoziationsgrade, wenn die Quotienten sehr gross sind ($q > 10^3$). In I s. 7–9 wurde bewiesen, dass in einer $n$-basischen Säure, wenn $H_{p+1}$ klein gegen $H_p$ ist, die Säure im Punkte $B = p$ hauptsächlich als Anion der Valenz $p$ existiert. Davon folgt, wenn die gesamten $q$ gross sind, dass die Säure im Neutralisationsgebiet Nr. $p$ hauptsächlich als Anionen der Valenzen $p-1$ und $p$ existiert. Die Gleichungen (2) und (4) liefern unter diesen Bedingungen

$$a_{p-1} + a_p = 1; \quad (p-1)\, a_{p-1} + p\, a_p = B$$

oder aufgelöst

$$a_{p-1} = p - B; \quad a_p = B - p + 1.$$

Die übrigen $\alpha$ sind in diesem Neutralisationsgebiet gleich 0. Im Neutralisationsgebiet Nr. $p$ gelten also die folgenden Formeln für $H_p$ und $K_p$

$$H_p = \frac{B - p + 1}{p - B}; \quad K_p = a_H \cdot \frac{B - p + 1}{p - B}. \tag{12}$$

In sehr einfacher Form können Formeln für die Dissoziationsgrade auch dann erhalten werden, wenn die $q$ gleich den Minimalwerten $q^0$ der Quotienten der thermodynamischen Dissoziationskonstanten sind.

Durch statistische Überlegungen ist von ADAMS abgeleitet worden, dass $q^0$ für eine zweibasische Säure grösser als 4 ist, und für eine dreibasische $q_1^0$ und $q_2^0$ beide grösser als 3.

Das statistische Verfahren von ADAMS lässt sich verallgemeinern, und man erhält für eine $n$-basische Säure die folgende Reihe der Minimal-Werte von $q^0$

$$q_1^0 = \frac{2\,n}{n-1}; \quad q_2^0 = \frac{3\,(n-1)}{2\,(n-2)} \cdots q_p^0 = \frac{(p+1)\,(n-p+1)}{p\,(n-p)}; \quad q_{n-1} = \frac{2\,n}{n-1}. \tag{13}$$

Aus der Formel für $q_p^0$ erhellt, wenn sie geschrieben wird;

$$q_p^0 = 1 + \frac{n+1}{p\,(n-p)}$$

dass für $p < \frac{n}{2}$ $q_p^0$ mit der Ordnungszahl abnimmt, für $p > \frac{n}{2}$ aber ansteigt und zwar so, dass $q_p^0 = q_{n-p}^0$. Das Minimum muss also erreicht werden für $p = \frac{n}{2}$, wenn $n$ gerade, und für $p = \frac{n-1}{2}$ und $\frac{n+1}{2}$, wenn $n$ ungerade ist, und beträgt im ersten Fall $\left(\frac{n+2}{n}\right)^2$, im letzten $\frac{n+3}{n-1}$. Für eine 4-basische Säure ist somit $q^0$ immer grösser als 2.25, für eine 5-basische immer grösser als 2.

Ferner kann aus (13) das Produkt der $q^0$ zu $n^2$ berechnet werden.

Durch Einsetzen der Werte von $q^0$ nach (13) in (9) kann der entsprechende Wert von $H_n$ ermittelt werden und daraus die übrigen $H$. Setzen wir $H_n = \frac{R}{n}$ und beachten, dass $P\,(q_{n-1}^0) = n^2$, ergibt sich

$$B + n\,(B-1)\,R + \frac{n\,(n-1)\,(B-2)}{2\,!}\,R^2 + \frac{n\,(n-1)\,(n-2)\,(B-3)}{3\,!}\,R^3 + \cdots = 0$$

oder

$$B\,(1+R)^n = n\,R\,(1+R)^{n-1}$$

woraus

$$R = \frac{B}{n-B}; \quad H_n = \frac{B}{n\,(n-B)}.$$

Es ergibt sich somit die folgende Reihe von den $H$

$$H_1 = \frac{Bn}{n-B}; \quad H_2 = \frac{B(n-1)}{2(n-B)}; \quad \cdots H_p = \frac{B(n-p+1)}{p(n-B)}; \quad H_n = \frac{B}{n(n-B)}. \tag{14}$$

Durch Substitution von diesen $H$-Werten in der aus (2), (4) und (5) leicht abzuleitenden Gleichung

$$B = a_0 [H_1 + 2 H_1 H_2 + \cdots n P(H_n)]$$

kann $a_0$ zu $\left(\frac{n-B}{n}\right)^n$ berechnet werden und daraus $a_1$, $a_2$ u. s. w. durch Heranziehung von (5) und (14). Es ergibt sich somit, dass die $a$ durch die Binomialentwicklung $\left(\frac{n-B}{n} + \frac{B}{n}\right)^n$ bestimmt sind, wo $a_n = \left(\frac{B}{n}\right)^n$ und die übrigen $a$ sich ihren Ordnungszahlen nach zwischen diesen einreihen.

Da, wie oben bewiesen, $\frac{\partial H_n}{\partial q}$ für alle $q$ negativ ist, muss der Betrag von $H_n$ nach (12) als ein Minimalwert betrachtet werden. Da ferner kaum kleinere Werte der $q$ als die nach (13) gegebenen beobachtet worden sind, kann der Betrag von $H_n$ nach (14) als eine *praktische* obere Grenze betrachtet werden, wodurch es möglich wird, den maximalen relativen Fehler zu bestimmen, womit $H_n$ nach der Grenzformel (12) ermittelt werden kann. Es wird erhalten

$$F_{H_n} = \frac{(n-1)(n-B)}{n(B-n+1)}. \tag{15}$$

Wenn die $q$ nicht gross sind, kann selbstverständlich nur am Ende des $n$:ten Gebiets $H_n$ ohne allzu grosse theoretische Fehler nach der Grenzformel berechnet werden. Setzen wir in (15) $B = n - \delta$, nimmt $F_{H_n}$ die Form

$$F_{H_n} = \frac{(n-1)\delta}{n(1-\delta)}$$

woraus folgt, dass $F_{H_n}$ bei konstantem $\delta$ mit der Basicität der Säure anwächst. Er bleibt aber offensichtlich immer kleiner als $\frac{\delta}{1-\delta}$ also für $\delta = 0{,}1$ immer kleiner als 11,1 %.

### Ionenstärken

Für die ionale Konzentration $\Gamma$ gilt definitionsgemäss

$$\Gamma = [\mathrm{Me}] + h + oh + c(a_1 + 4a_2 + \cdots n^2 a_n).$$

Nun ist aber $[\mathrm{Me}] + h + oh = Bc + 2oh$. Wird der Ausdruck für $B$ nach (4) in obiger Gleichung eingesetzt und $\Gamma - 2oh$ gleich $\Phi$ gesetzt, wird erhalten

$$\Phi = 2c\left(a_1 + 3a_2 + \cdots \frac{n^2+n}{2} a_n\right). \tag{16}$$

Aus (4) und (16) kann abgeleitet werden

$$\Phi = 2\,Bc\,\frac{a_1 + 3\,a_2 + \cdots \frac{n^2+n}{2}\,a_n}{a_1 + 2\,a_2 + \cdots n\,a_n}.$$

Substitution von $H$ statt $a$ vermittels (5) führt zu

$$\Phi = 2\,Bc\,\frac{H_1 + 3\,H_1 H_2 + \cdots \frac{n^2+n}{2}\,P(H_n)}{H_1 + 2\,H_1 H_2 + \cdots n\,P(H_n)}. \qquad (17\,\mathrm{a})$$

Da $H_1$ als Faktor im Zähler und Nenner vorkommt, wird $\Phi$ laut (17 a) als Funktion von $Bc$ und den $n-1$ letzten $H$ bestimmt.

Gl. (2) (4) (5) und (16) können folgendermassen kombiniert werden

$$\frac{(n+1)\,Bc - \Phi}{(n-B)\,c} = \frac{(n-1)\,H_1 + 2\,(n-2)\,H_1 H_2 + \cdots (n-1)\,P(H_{n-1})}{n + (n-1)\,H_1 + (n-2)\,H_1 H_2 + \cdots P(H_{n-1})} \qquad (17\,\mathrm{b})$$

wodurch $\Phi$ als Funktion von den $(n-1)$ ersten $H$ dargestellt wird. Da sowohl in (17 a) wie in (17 b) der Quotient der Koeffizienten entsprechender Terme in Zähler und Nenner mit der Ordnungszahl ansteigt, müssen die partiellen Ableitungen $\frac{\partial \Phi}{\partial H}$, wenn die $n-1$ letzten $H$ als unabhängige Veränderliche gewählt werden, positiv sein, hingegen negativ, wenn die $n-1$ ersten es werden. Aus (17 a) und (17 b) ist ferner ersichtlich, dass der Minimalwert von $\Phi$ gleich $2\,Bc$ und der Maximalwert gleich $(n+1)\,Bc$ ist.[1]

Eine Frage von Bedeutung für die Berechnung von $\Phi$ und somit auch von $\Gamma$ ist, wie $\Phi$ bei konstanten $B$ und $c$ geändert wird, wenn die Quotienten $q$ geändert werden. Für die Analyse dieses Problems ziehen wir die Gleichungen (2) (4) und (16) heran.

Es wurde oben bewiesen, dass wenn eines der $q$, sei es $q_r$, wachsen darf unter Konstantbleiben der übrigen, die Inkremente der $a$ ihres Zeichens nach in drei Gruppen aufgeteilt werden können, zuerst eine negative, dann eine positive, deren erstes Glied $\Delta\,a_u$ sei und letztes $\Delta\,a_v$, und schliesslich wieder eine negative. Da $\Delta\,a_0$ negativ ist, hat man also

$$\sum_1^n \Delta\,a_p > 0; \quad \sum_1^n p\,\Delta\,a_p = 0$$

woraus durch Subtraktion erhalten wird

$$\sum_2^n (p-1)\,\Delta\,a_p < 0.$$

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[1] Die Idee, die ionale Konzentration durch die $H$ auszudrücken ist von BJERRUM und UNMACK übernommen worden. Diese Verfasser verwenden für die Berechnung von Ionenstärken für Zitronensäure durch sukzessive Approximation eine Formel, wo die drei Konstanten $K$ der Säure eingehen, was aber überflüssig ist, da man mit zwei auskommt.

Ferner gibt Differentiierung von (16)

$$\frac{\Delta \Phi}{2c} = \sum_1^n \frac{p(p+1)}{2} \Delta a_p;$$

subtrahiert man davon $\sum\limits_1^n p \Delta a_p$, resultiert

$$\sum_2^n \frac{p(p-1)}{2} \Delta a_p.$$

Nun ist aber $\sum\limits_2^n \Delta a_p > 0$, und wird diese Ungleichung von $\sum\limits_2^n (p-1) \Delta a_p$ subtrahiert, entsteht $\sum\limits_3^n (p-2) \Delta a_p < 0$. Wird nun $\sum\limits_2^n (p-1) \Delta a_p$ von $\sum\limits_2^n \frac{p(p-1)}{2} \Delta a_p$ subtrahiert, resultiert $\sum\limits_3^n \frac{(p-1)(p-2)}{2} \Delta a_p$. In dieser Weise wird fortgesetzt, bis alle Terme der ersten negativen Gruppe verschwunden sind, da von $\frac{\Delta \Phi}{2c}$ die Reihe

$$\sum_u^n \frac{(p-u+2)(p-u+1)}{2} \Delta a_p$$

übrig bleibt und von $\Delta B$

$$\sum_u^n (p-u+1) \Delta a_p,$$

welche letzte Summe negativ sein muss, da sie durch wiederholte Subtraktionen von positiven Quantitäten von 0 entstanden ist.

Der Quotient der Koeffizienten für den letzten positiven Term $\Delta a_v$ in der $\Delta \Phi$-Reihe und der $\Delta B$-Reihe ist $\frac{v-u+2}{2}$, eine Zahl, die offenbar grösser als 1 ist, und der Quotient der Koeffizienten für den ersten negativen Term der letzten negativen Gruppe $\frac{v-u+3}{2}$. Da die Quotienten bei wachsender Ordnungszahl wachsen, gilt

$$\sum_u^v \frac{(p-u+1)(p-u+2)}{2} \Delta a_p < \frac{v-u+2}{2} \sum_u^v (p-u+1) \Delta a_p$$

und

$$\sum_{v+1}^n \frac{(p-u+1)(p-u+2)}{2} \Delta a_p < \frac{v-u+3}{2} \sum_{v+1}^n (p-u+1) \Delta a_p$$

und also

$$\sum_u^n \frac{(p-u+1)(p-u+2)}{2} \Delta a_p < \frac{v-u+2}{2} \sum_u^v (p-u+1) \Delta a_p + \\ + \frac{v-u+3}{2} \sum_{v+1}^n (p-u+1) \Delta a_p.$$

Da $\sum\limits_{u}^{n}(p-u+1)\,\Delta a_p$, wie soeben betont, negativ ist, muss dies auch a fortiori für das rechte Membrum der letzten Ungleichung gelten und somit auch für das linke. Nun ist dieses aber durch wiederholte Subtraktion von negativen Grössen von $\frac{\Delta \Phi}{2c}$ entstanden, weshalb diese Grösse sich als eine Summe von lauter negativen Quantitäten darstellt und somit negativ sein muss. Bei Übergang zu Limes erhalten wir also $\frac{\partial \Phi}{\partial q_r} < 0$ für die ganze $B$-Skala und für alle $q$ mit Indices von 1 bis $n-1$.

Wenn wir also zwei Säuren derselben Basicität vergleichen, die zu demselben $B$-Wert neutralisiert sind und zwar so, dass die Totalkonzentrationen dieselben bleiben, dann hat $\Phi$ einen höheren Betrag in derjenigen Säure, wo sämtliche $q$ kleinst sind.

Wenn die $q$ sehr gross werden, erhält $\Phi$ seinen Minimalbetrag, der sich leicht berechnen lässt.

Für das Neutralisationsgebiet Nr. $\nu$ ist wie oben s. 358 bewiesen wurde,

$$a_{\nu-1} = \nu - B; \quad a_\nu = B - \nu + 1.$$

Einsetzen von diesen Werten in (16) liefert

$$\Phi = \nu\,(2\,B - \nu + 1)\,c. \tag{18}$$

Bei grossen $q$ ist somit $\Phi$ im ersten Gebiet gleich $2\,Bc$, im zweiten gleich $(4\,B-2)\,c$, im dritten gleich $6\,(B-1)\,c$.

Eine sehr einfache Form erhält die Formel für $\Phi$ auch für den Fall, dass die Quotienten $q$ nach (13) bestimmt sind.

Werden in Gl. (17 a) die entsprechenden $H$-Werte nach (14) substituiert, gelangt man, wenn $\frac{B}{n-B}$ gleich $P$ gesetzt wird, zu dem folgenden Ausdruck

$$\Phi = 2\,Bc\,\frac{1 + \frac{3\,(n-1)}{2!}P + \frac{6\,(n-1)\,(n-2)}{3!}P^2 + \cdots \frac{n+1}{2}P^{n-1}}{1 + \frac{2\,(n-1)}{2!}P + \frac{3\,(n-1)\,(n-2)}{3!}P^2 + \cdots P^{n-1}} =$$

$$= 2\,Bc\,\frac{(1+P)^{n-1} + \frac{n-1}{2}P\,(1+P)^{n-2}}{(1+P)^{n-1}} =$$

$$= Bc\,\frac{P\,(n+1)+2}{1+P} = \frac{(n-1)\,B^2 + 2\,n\,B}{n}\cdot c. \tag{19}$$

Betrachten wir die Werte von $q$ nach (13) als Minimalwerte, kann aus (18) und (19) der maximale relative Fehler $F_\Phi$ ermittelt werden, womit $\Phi$ im $n$:ten Gebiet nach der Grenzformel (18) berechnet werden kann. Es wird erhalten

$$F_\Phi \doteq \frac{(n-1)(n-B)^2}{n^2(2B-n+1)}. \tag{20}$$

Es geht aus dieser Formel hervor, dass der Fehler im $n$:ten Gebiet bei wachsendem $B$ abnimmt und am Ende des Gebiets hinreichend klein werden kann, damit $\Phi$ mit genügender Genauigkeit nach (18) berechnet werden kann.

Setzen wir in (20) $B = n - \delta$, nimmt $F_\Phi$ die Form

$$F_\Phi = \frac{(n-1)\delta^2}{n^2(n-2\delta+1)}.$$

Bei konstantem $\delta$ sinkt, wie sofort einzusehen, der Betrag von $F_\Phi$ mit wachsendem $n$, weshalb der Fehler am grössten für eine 2-basische Säure ist, wo er für $\delta = 0{,}1$ 0,089 % beträgt.

Die Möglichkeit sowohl $\Phi$ wie $K_n$ am Ende des $n$:ten Gebiets nach einfachen Grenzformeln zu berechnen und somit auch Aktivitätskoeffizienten, führt zur Regel, beim Berechnen von Dissoziationskonstanten aus potentiometrischen Messungen immer mit der letzten Konstante zu beginnen, wie in I näher für zwei- und dreibasische Säuren erläutert wurde.

LITERATUR. **Söderbäck,** Arkiv Kemi, Mineral. Geol. Bd *11 A* N:o 8 (1934). — **Auerbach** u. **Smolczyk,** Z. physik. Ch. *110* 65 (1924). — **Bjerrum** u. **Unmack,** Elektrometrische Messungen mit Wasserstoffelektroden. Det Kgl. Danske Vidensk. Selsk. Mathem. fysik. Meddel. IX 1 (1929). — **Adams,** Journ. Amer. Chem. Soc. *38,* 1503 (1916).

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# The reaction of diethyl malonate with acid anhydrides. A new synthesis of β-diketones of the type $RCOCH_2COR$

By Arne Brändström

Various methods for synthezing β-diketones of the type $RCOCH_2COR$ are known (1—5). However, none has been entirely satisfactory for the preparation of certain β-diketones. The present paper describes a new method that appears to be quite general and which makes use of readily available starting materials.

In 1944 BRESLOW, BAUMGARTEN and HAUSER (6) described a method of preparing β-keto esters of the type $RCOCH_2COOC_2H_5$ from acylated ethyl *t*-butyl malonates by acidolysis with *p*-toluenesulphonic acid. They also reported that an attempt to prepare certain β-ketoesters from diethyl malonates instead of the ethyl *t*-butyl malonates failed. However, COHEN (7) has reported that there is a great difference between the acidolysis catalysed by *p*-toluenesulphonic acid and that catalysed by a basic agent such as sodium or magnesium acetate. In the acid catalysed acidolysis of esters, the tertiary alcohol ester is most reactive, the secondary is less reactive, and, under comparable conditions, the primary alcohol esters are inert. In reactions which proceed by coordination of a basic reagent at the carbonyl carbon atom of an ester the inverse order of reactivity is found. This makes it likely that a basic reagent might catalyse the reaction of acylated diethyl malonates to yield β-ketoesters.

REEDER AND LESCISIN (8) showed that a base catalysed acidolysis of compounds of this type is possible and prepared certain β-diketones by acidolysis of β-diketoesters by means of an organic acid and sodium acetate as a catalyst. The same authors (9) also described a method of preparing β-diketones from a β-ketoester and an anhydride according to the reaction:

$$\begin{aligned}&RCOCHR'COOR'' + (R'''CO)_2O \rightarrow RCOCR'(R'''CO)COOR'' + R'''COOH \rightarrow\\&RCOCHR'COR''' + R'''COOR'' + CO_2\end{aligned}$$

A small amount of magnesium oxide was found to be a very efficient catalyst.

DICKMANN AND STEIN (10) indicated that a similar reaction might be possible using diethyl malonate and an acid anhydride and showed that ethyl diacetylacetate was formed from the reaction of diethyl malonate with acetic anhydride using a small amount of sodium acetate as a catalyst. The same reaction has been used by ERRARA (11) to prepare *peri*-naphtindandione from diethyl malonate and the anhydride of 1.8-naphthalenedicarbonic acid using $ZnCl_2$ as a catalyst, but the reaction has not been extended further than to some other naphthalene derivatives (12—15).

In the present investigation β-diketones have been obtained in good yields from diethyl malonate and an acid anhydride using a small amount of magnesium oxide as a catalyst. As WIDEQVIST (16) has found that traces of copper acetate catalyse decarboxylation very well so it also was added to the reaction mixture.

The progress of the reaction:

$$CH_2(COOC_2H_5)_2 + (RCO)_2O \rightarrow RCOCH(COOC_2H_5)_2 + RCOOH \rightarrow$$
$$RCOCH_2COOC_2H_5 + RCOOC_2H_5 + CO_2$$
$$RCOCH_2COOC_2H_5 + (RCO)_2O \rightarrow (RCO)_2CHCOOC_2H_5 + RCOOH \rightarrow$$
$$RCOCH_2COR + RCOOC_2H_5 + CO_2$$

can be followed by the evolution of carbon dioxide or by the formation of the ethyl ester, which can be distilled off from the mixture.

A present investigation deals with attempts to use alkylated diethyl malonates in the reaction and the possibilities for the preparations of β-ketoesters by a similar reaction.

## Experimental

### Preparation of acid anhydrides

All acid anhydrides used in this investigation were prepared by refluxing a mixture of 2 moles of the acid with 1.1 moles of thionyl chloride for 40 hours and fractionating the resulting product. After a forerun of the acid or the acid chloride the anhydride goes over. In this way yields of over 90 % of the anhydrides were obtained.

### Preparation of β-diketones

A mixture of 1 mole of pure diethyl malonate, 2 moles of the acid anhydride, 0.2 g of magnesium oxide, and 0.1 g of copper acetate was heated in a round-bottomed flask fitted with a 30 cm Widmer column. The heating was so regulated that only the ethyl ester was distilled over. The reaction was considered as complete when no more ester could be obtained, which usually was the case when over a 90 % yield thereof had been obtained. This operation takes about 2–3 hours.

After cooling, the mixture was acidified with 10 ml of 10 % sulphuric acid and then steam distilled and extracted whith ether in a continuously operating apparatus (17). The ether extract was dried with sodium sulphate and fractionated. After a forerun containing ether and acids the β-diketone comes over in a yield of 50–65 %. The β-diketones whose boiling points are to close to that of diethyl malonate or the corresponding β-ketoester to permit removal of the last traces thereof by distillation were purified over the copper derivative. In this way the following β-diketones were obtained: *Dipropionylmethane* b. p. 174°–175°, *diisobutyrylmethane* b. p. 61°–62°/8 mm, *dibutyrylmethane* b. p. 81°–81.5°/8 mm, and *3.7-diethyl dibutyrylmethane* b. p. 110°–111°/9 mm.

The yields are probably not the maximum yields obtainable, and in special cases it may be better to add the diethyl malonate dropwise to the anhydride.

The expenses in this research have been defrayed by a grant from *Statens Naturvetenskapliga Forskningsråd* which is gratefully acknowledged.

## SUMMARY

A general method for the preparation of β-diketones of the type $RCOCH_2$ COR from diethyl malonate and an acid anhydride has been described.

An improved method of preparing acid anhydrides has been indicated.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, May 1951.

REFERENCES. 1. **R. Levine, J. A. Conroy, J. T. Adams** and **C. R. Hauser,** J. Am. Chem. Soc. *67,* 1510 (1945). — 2. **E. Man, F. Swamer** and **C. R. Hauser,** ibid. *73,* 901 (1951). — 3. **F. Joung, F. Frostick. J. Sanderson** and **C. R. Hauser,** ibid. *72,* 3635 (1950). — 4. **F. Swamer** and **C. R. Hauser,** ibid. *72,* 1352 (1950). — 5. **A. Magnani** and **S. M. Mc Elvain,** ibid. *60,* 813 (1938). — 6. **D. Breslow, E. Baumgarten** and **C. R. Hauser,** ibid. *66,* 1286 (1944). — 7. **S. Cohen,** ibid. *66,* 1395 (1944). — 8. **W. Reeder** and **G.Lescisin.** U. S. Patent 2.369.250 (1945) C. A. *40,* 901 (1946). — 9. **W. Reeder** and **G. Lescisin,** U. S. Patent 2.395.012 (1946) C. A. *40,* 3130 (1946). — 10. **W. Dickmann** and **R. Stein,** Ber. *37,* 3378 (1904). — 11. **G. Errara,** Gazz. chim. ital. *41 : 1,* 191 (1911). — 12. **F. Noto,** ibid. *45 : 2,* 127 (1915). — 13. **E. Calderaro,** ibid. *45 : 2* 131 (1915). — 14. **M. Freund** and **K. Fleischer,** Ann. *402,* 79 (1914). — 15. **S. Gabriel,** Ber. *17,* 1396 (1889). — 16. **S. Wideqvist,** Arkiv f. Kemi, Min. och Geol. *26 A* N:o 16 (1948). — 17. **A. Vogel,** *Practical Organic Chemistry,* 223 (1948).

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# The homogeneity of a reported peptide fraction[1]

By I. Gordon Fels and Arne Tiselius

With 2 figures in the text

The report has recently been made by Borsook and co-workers (1, 2) of a peptide fraction isolated from numerous tissues as well as Witte peptone. The identity of the material obtained from the various sources is evidenced by its similar chromatographic behaviour on starch before and after precipitation by picric, flavianic or trichloracetic acids and subsequent removal of the precipitating agent. In addition, the amino acid composition is not demonstrably different prior to or after the precipitation of the fraction in every case. The substance has been designated as Peptide A.[2]

Inasmuch as the peptide fraction appears to be metabolically active (1), work was undertaken in this Laboratory to investigate the material with particular emphasis upon its homogeneity. Furthermore, it was important to have a test substance to evaluate different techniques which have been used for the separation of small peptides. In view of the apparent large size of Peptide A (2), the latter is of particular interest. The present brief report presents results obtained from filter paper chromatography.

Witte peptone (Rostock) was chosen as the representative source of Peptide A, because of its general availability. The preparation (2) was slightly modified to give what we believe to be a better looking product. After precipitation with 10 % trichloracetic acid (final volume), the material was centrifuged, supernatant discarded, and the precipitate vigorously extracted three times with 100 ml ether. The precipitate was then redissolved in 75 ml of distilled water and freeze dried with alcohol and solid $CO_2$. From 8 g of peptone, 2.2 g of a white, fluffy substance were obtained. Other brands of peptone were tried (Difco Bacto-Peptone and Grave Peptone) but the yields were very unsatisfactory. Large amounts of material could be obtained also from Grave Witte Peptone (Stockholm).

Peptide A was obtained by chromatography on starch of the peptides obtained from Witte peptone. These will be designated as the Rostock peptides. The procedure was identical to that used by the previous authors (2), and will not be given here. However, since the identity of the material rests largely upon its chromatographic behavior on starch, in particular, its relationship to the leucines which are the first amino acids emerging from the column, the results of a

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[1] This work has been undertaken with the joint sponsorship of the American Cancer Society, Committee on Growth and the American Scandinavian Foundation.

[2] It has also been referred to as the "tissue peptide of Borsook".

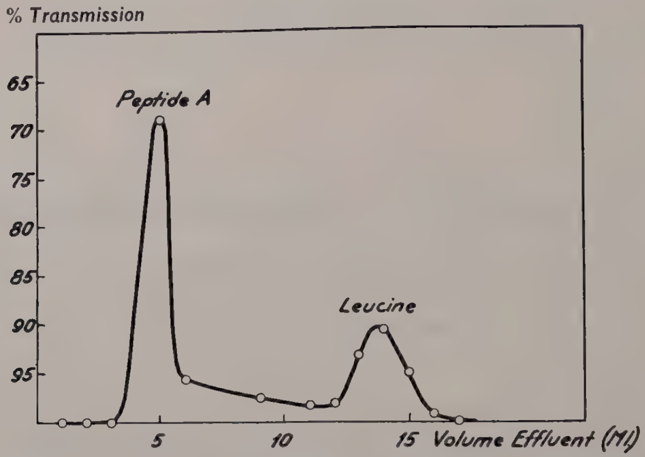

Fig. 1. The behaviour of Peptide A on starch. 4 mg of peptide admixed with 0.2 mg leucine added to column, 9 mm × 30 mm, containing 19.1 g starch; total $H_2O$ content — 20 %, solvent: *n*-propyl alcohol, *n*-butyl alcohol, 0.1 N HCl (2:1:1). One ml fractions collected; 0.2 ml analyzed.

chromatograph with admixed leucine is given in Figure 1. This is almost identical with that obtained by the previous workers. Non-specific properties identifying this material with Peptide A also might be mentioned. The substance gives a purple biuret test, an insoluble picrate and flavianate.

The homogeneity of Peptide A was tested by descending filter paper chromatography using as solvent, *n*-propyl alcohol, *n*-butyl alcohol and 0.1 *N* HCl (2:1:1) on Munktell OB filter paper. Inasmuch as this solvent mixture is the same as that used with the starch column in obtaining Peptide A, a comparison could be made between the two adsorbents. It was for this reason that leucine was added to the filter paper as a control. After 1–2 hours, the chromatogram was dried and sprayed with ninhydrin solution in the usual manner, and heated for 1/2 hour at 100° C in a dry oven. The results are given in Figure 2, demonstrating that Peptide A is a mixture of at least four components. The entire experiment was repeated with essentially the same results.

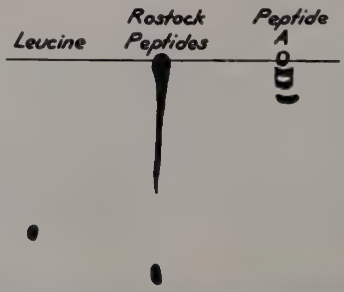

Fig. 2. Paper chromatography of Peptide A. Solvent: *n*-propyl alcohol, *n*-butyl alcohol, 0.1 N HCl; time—1 hr.

Also apparent from Figure 2 is the fact that the behaviour of filter paper and starch as adsorbents is considerably different. Whereas on starch, Peptide A precedes leucine, on paper the converse is true. This difference is particularly interesting in view of the close chemical relationship between the two substances. This was again confirmed by passing a mixture of leucine and Peptide A through a column of paper pulp ("solka floc"). The same solvent system was used as previously, and fractions were collected without application of pressure. Leucine emerged in Fractions 19–22, whereas Peptide A appeared in Fraction 37. The substances were identified by determining separately the fraction at which each makes its appearance. No attempt was made to fractionate Peptide A, although it would appear to be possible. Attempts to fractionate the Rostock peptides on paper have thus far been unsuccessful. From Figure 2, the indications are that this is not possible with the present solvent system.

Finally, it would appear that the behaviour of starch toward the larger peptides is non-discriminant and extreme. A calculation from Figure 3 of BORSOOK's paper (2), corresponding to Figure 1 in this report, indicates that about 45 % of the material is retained on the starch column. The remainder, Peptide A, is apparently not adsorbed at all. The results obtained here are in agreement with Stein and Moore (3) who have stated that. ". . . at the point of emergence of the advancing front of solvent, which is about 6 cc after the sample has been added to a column 0.9 × 30 cm, there will emerge all the substances which are not adsorbed at all by starch. A peak at this position, therefore, would, in all likelihood, be a mixture of substances". These authors, as well as LI and PEDERSEN (4) have found starch unsuitable to fractionate the higher peptides efficiently.

## SUMMARY

1. A peptide fraction previously reported present in numerous tissues as well as Witte peptone has been found to be a mixture.

2. The behavior of starch as an adsorbent for higher peptides is different from that of filter paper.

3. With the present solvent system, starch is not an efficient adsorbent in the fractionation of higher peptides.

BIBLIOGRAPHY. 1. **Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley G.,** and **Lowry, P. H.,** *J. Biol. Chem. 174*, 1041, 1948. — 2. *Ibid. 179*, 705, 1949. — 3. **Stein, W. H.,** and **Moore, S.,** *Symp. Quant. Biol.* XIV, 179, 1950. — 4. **Li, C. H.,** and **Pedersen, K. O.,** *Ark. f. Kemi* (1), *61*, 533, 1950.

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# The crystal structure of $CsSb_2F_7$

By ANDERS BYSTRÖM and KARL-AXEL WILHELMI

With 1 figure in the text

In a previous paper (1) the crystal structure of a series of isomorphous compounds with the general formula $RSb_4F_{13}$ (R = K, Rb, Cs, $NH_4$, Tl) was reported. In this structural type four $SbF_3$ molecules are arranged around the thirteenth F atom. The Sb—F distances within the $SbF_3$ molecule are about 2.0 Å and the distances to the central thirteenth F atom about 3 Å. Thus no equilization of the Sb—F bonds in the $Sb_4F_{13}^-$ group occurs in this case and the individuality of the $SbF_3$ molecules is preserved. However, this is not the case in the $Sb_2F_7^-$ complex in $CsSb_2F_7$ as will be seen from this paper.

## Preparation and analyses of the compound

$CsSb_2F_7$ was prepared by dissolving $CsHCO_3$ and $Sb_2O_3$ in the proportions Cs : Sb = 1 : 2 in an excess of dilute hydrofluoric acid and crystalizing at room temperature. Two types of crystals were observed: thin plates and rather elongated prisms, but as the powder photographs of the two kinds of crystals were identical, they both consisted of the same compound. The crystals were analysed and the following values obtained which agree with the formula $CsSb_2F_7$:

| | | | | | | |
|---|---|---|---|---|---|---|
| Sb (bromate titration): | 47.6 % | calculated | for | $CsSb_2F_7$: | 47.90 |
| Cs (as $Cs_2PtCl_6$): | 25.9 » | » | » | » | : 26.1 |

## Determination of unit cell and space group

Rotation and Weissenberg photographs (Cu—$K$ radiation) showed that the crystals were monoclinic, and from powder photographs (Cr—$K$; $\lambda_a = 2.2909$ Å) the following cell dimensions were derived:

$$a = 19.32\ \text{Å} \qquad b = 6.89\ \text{Å} \qquad c = 5.58\ \text{Å} \qquad \beta = 90°.$$

The density was found to be 4.54. If the cell content is assumed to be 4 $CsSb_2F_7$, the density calculated from the x-ray data will be 4.55, in perfect agreement with the observed value.

The following reflections were observed

$h\,k\,l$ with $h + k = 2\,n$
$h\,k\,0$ » $h + k = 2\,n$
$h\,0\,l$ » $h = 2\,n$ and $l = 2\,n$
$0\,k\,l$ » $k = 2\,n$.

The habit of the crystals showed holohedral symmetry. Attempts to etch the crystal faces were made but it was difficult to find a suitable etching agent. However, in one favourable case etch-figures, lying on the face (100), were obtained with moist air as etching agent. As these etch-figures also showed holohedral symmetry, there was no reason to doubt that the space group was $C_{2h}^6 - C\,2/c$.

The x-ray examination showed that the *a*-axis was perpendicular to the large face of the thin crystal plates. As this habit of the crystals might indicate a layer lattice, attempts were made to obtain the cleavage along (100). However, this was not possible and only irregular fragments were obtained. It seemed that the cohesion of the crystals in the [100] direction was as good as in the other directions. In the prismatic crystals, [001] was orientated along the prism-axis.

## Positions of the metal atoms

Only one arrangement of the metal atoms gave satisfactory agreement between observed and calculated intensities. The following parameter values were obtained both from a trial and error process and from two-dimensional Fourier syntheses in the $x\,y$- and $x\,z$-planes:

$C_{2h}^6 - C\,2/c$ $(000;\ \tfrac{1}{2}\,\tfrac{1}{2}\,0)\ +$

4 Cs in 4 (*e*): $\pm\,(0\,y\,\tfrac{1}{4})$ $\quad y = 0.230 \pm 0.003$

8 Sb in 8 (*f*): $\pm\,(x\,y\,z);\ \pm\,(\bar{x}\,y\,\tfrac{1}{2} - z);\ x = 0.1694 \pm 0.0015;$
$y = -0.239 \pm 0.003;\ z = 0.283 \pm 0.003.$

## The positions of the fluorine atoms

As the best Weissenberg photographs were obtained with [001] as rotation axis, the arrangement of the fluorine atoms in the $x\,y$ plane was considered first. Several minor discrepancies between observed intensities and those calculated taking into consideration the influence of the metal atoms only, indicated that it might be possible to derive, or at least confirm, the positions of the fluorine atoms from the intensities in spite of the unfavourable relation between the scattering powers of these atoms and the metal atoms (see table below).

The symmetry of the space group $C_{2h}^6$ allows of only four-fold and eight-fold positions. Of the four-fold positions, 4 (*a*) and 4 (*b*) could not possibly be occupied by F atoms, because for these positions Cs–F distances of only 2.1 and 2.3 Å, respectively, are obtained. Space considerations showed also that either 16 or 24 of the F atoms must be placed in the general position. Thus one of the two cases A and B must apply:

| A | B |
|---|---|
| 4 F in 4 (*c*), 4 (*d*), or 4 (*e*) | 12 F in 4 (*c*) + 4 (*d*) + 4 (*e*) |
| 24 F in $3 \cdot 8$ (*f*) | 16 F in $2 \cdot 8$ (*d*) |

Attempts to derive the F-positions from the Patterson projection $x\,y\,0$ were not successful. As the signs of the $F$-values of all reflexions but a few weak ones could be established, the metal atom positions being known, a Fourier summation $h\,k\,0$ was also tried but it was found that the minor peaks obtained in this projection could not correspond to the positions of the F atoms.

However, some $h\,k\,0$ reflexions were visible, though the sum of the structure factors of the metal atoms was equal to zero. Further, a few reflexions were not visible though the magnitude of the metal atom scattering was sufficiently high. Thus it seemed possible to derive the positions of the fluorine atoms from these discrepancies.

Obviously, the fluorine atoms have a comparatively small influence on the strong reflexions $h\,k\,0$ (those with $h = 6\,n$). Therefore, approximately correct absolute $F$-values for the weak reflexions could be obtained by multiplying the values of $F_{\text{obs}}$ by the quotient $K = \frac{F_{\text{Met}}}{F_{\text{obs}}}$ for the nearest strong reflexion, $F_{\text{Met}}$ being the value of the structure factor for the metal atoms.

An elaborate trial and error process indicated that of the alternatives for the fluorine atom positions — three eight-fold positions + one four-fold position or two eight-fold position + three four-fold position — only the former led to consistent results. The following arrangement in the $x\,y$ projection was obtained:

| | $2\pi x$ | $2\pi y$ |
|---|---|---|
| 4 F in 4 (*c*) or 4 (*d*): | 90° | 90° |
| 8 $F_2$ in 8 (*e*): | 25° | 210° |
| 8 $F_3$ » » | 38° | 0° |
| 8 $F_4$ » » | 60° | 105° |

The table below shows that with this arrangement the influence of the fluorine atoms is of the right magnitude for most of the weak or absent $h\,k\,0$ reflexions. The table shows only reflexions with $k \leq 3$, because the influence of the fluorine atoms on the other reflexions is too small to be considered. $F_{\text{Met.}}$ and $F_{\text{Fluor.}}$ denote the sum of the structure factors of the metal atoms and fluorine atoms respectively, and $K \cdot F_{\text{obs}}$ the value of $F_{\text{obs}}$ multiplied by a factor derived as shown above.

As will be seen, almost all the discrepancies between the values of $K \cdot F_{\text{obs.}}$ and $F_{\text{Met.}}$ disappear when the values of $F_{\text{Fluor.}}$ are added ($= \Sigma F$ in the table), thus indicating that the suggested $x$ and $y$ parameters of the fluorine atoms are probably correct, though of course the errors in the parameter values may be considerable, especially in the $y$-direction.

| $h\,k\,l$ | $K \cdot F_{obs.}$ | $F_{Met.}$ | $F_{Fluor.}$ | $\Sigma F$ |
|---|---|---|---|---|
| 3 1 0 | – | –1 | –25 | –26 |
| 5 1 0 | – | 37 | –37 | 0 |
| 7 1 0 | 60 | 31 | 35 | 66 |
| 9 1 0 | 70 | –1 | 78 | 77 |
| 11 1 0 | 40 | 31 | 25 | 56 |
| 13 1 0 | – | 22 | –49 | –27 |
| 15 1 0 | 60 | 0 | –40 | –40 |
| 2 2 0 | 70 | –2 | 95 | 93 |
| 4 2 0 | 70 | –31 | –60 | –91 |
| 8 2 0 | 50 | 26 | 1 | 27 |
| 10 2 0 | – | –52 | 52 | 0 |
| 14 2 0 | – | 37 | 12 | 49 |
| 1 3 0 | – | –94 | 55 | –41 |
| 3 3 0 | 100 | 4 | –54 | –50 |
| 5 3 0 | 100 | –94 | –28 | –122 |
| 7 3 0 | 100 | –78 | 12 | –66 |
| 9 3 0 | – | 3 | 10 | 13 |
| 11 3 0 | 60 | –83 | 28 | –55 |
| 13 3 0 | 80 | –63 | –10 | –73 |
| 15 3 0 | – | –1 | –38 | –39 |

Considerations of the interatomic distances show that approximate values for the $z$-parameters can be obtained if it can be decided whether the four-fold position is 4 ($c$): $\frac{1}{4}\frac{1}{4}0$ or 4 ($d$): $\frac{1}{4}\frac{1}{4}\frac{1}{2}$. In the former case the $z$-parameter of three eight-fold positions must be approximately 0.3, 0.2 and 0.45, in the latter case 0.2, 0.3, and 0.15. The two structures which correspond to them do not differ in principle but only in the length of the Sb–F bonds in the Sb–F–Sb bridge, which is 1.97 Å in the former structure and 2.22 Å in the latter one. We attempted to decide between these two possibilities by considering the intensities of the $h\,0\,l$ reflexions. (The Fourier syntheses is of little value, because of the difficulty in estimating the intensities with sufficient accuracy.) However, in these calculations similar difficulties were met with as in the case of $TlSb_4F_{13}$ (1). There seems to be some effect which is not taken into consideration in the ordinary intensity formula, and which probably originates in some disturbance in the symmetry of the electron arrangement of the heavy atoms. It is interesting to note that WATANABÉ, ATOJI and OKAZAKI (2) found similar effects for $Co(NH_3)_6TlBr_6$ but not for the isomorphous compound $Co(NH_3)_6TlCl_6$ and that they noted that the discrepancies could not be eliminated by varying the parameters. $Pb_2O_3$ (3 and 4) also showed discrepancies, which could not be reconciled by a parameter variation. It seems that the effect is apparent

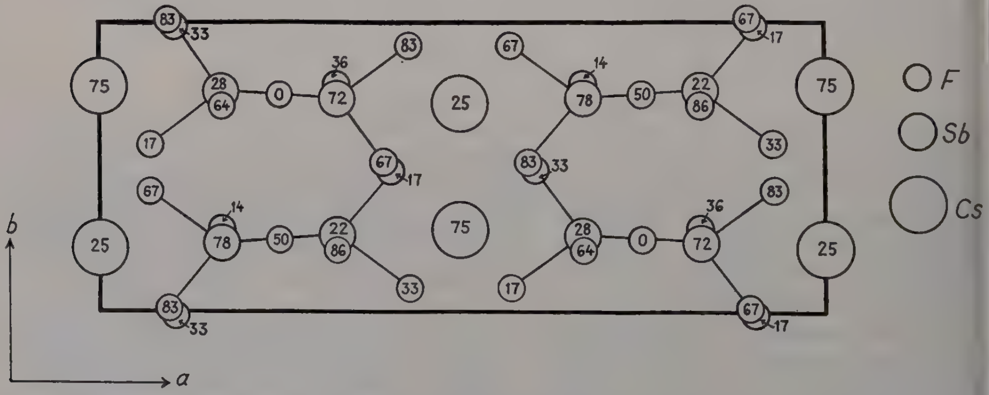

Fig. 1. $CsSb_2F_7$. Projection on (001). The figures denote the height of the atoms in percentages of $c$. Superimposed atoms are symmetrically displaced.

only when at least two kinds of heavy atoms (there are two different lead atom positions in $Pb_2O_3$) are present in the structure.

The distance Sb–F in an $SbF_3$ group is probably close to 2.0 Å (1 and 5). As there are four Sb–F bonds in $CsSb_2F_7$, it is reasonable to assume that the Sb–F bonds in the Sb–F–Sb bridge are 0.1–0.2 Å longer than in an $SbF_3$ group. Thus the four-fold position of the fluorine atoms is probably 4 (*d*) (Sb–F = 2.22 Å) and not 4 (*c*) (Sb–F = 1.97 Å). A variation of the *z*-parameter for the other three F positions indicated that the best general agreement was obtained with the following parameter values:

$C_{2h}^6 - C\ 2/c\ (000:\ \tfrac{1}{2}\tfrac{1}{2}0)\ +$

4 Cs in 4 (*e*): $\pm\ (0\,y\,\tfrac{1}{4})$ $\quad y = 0.230 \pm 0.003$

8 Sb in 8 (*f*): $\pm\ (x\,y\,z)$;

$\pm\ (\bar{x}\,y\,\tfrac{1}{2}-z)$; $\quad x = 0.1694 \pm 0.0015 \quad y = -0.239 \pm 0.003 \quad z = 0.283 \pm 0.003$

4 $F_1$ in 4 (*d*): $\tfrac{1}{4}\tfrac{1}{4}\tfrac{1}{2};\ \tfrac{3}{4}\tfrac{1}{4}0$;

8 $F_2$ in 8 (*f*): $\quad x = 0.0695 \quad y = 0.583 \quad z = 0.167$

8 $F_3$ in 8 (f): $\quad x = 0.105 \quad y = 0.00 \quad z = 0.333$

8 $F_4$ in 8 (*f*): $\quad x = 0.167 \quad y = 0.292 \quad z = 0.139$

The maximal errors in the F parameters are probably ± 0.01 for the *x*-parameters and ± 0.03 for the *y*-parameters. It is hardly possible to estimate the errors in the *z*-parameters from the intensity calculations, but the errors in these parameters are probably of the same magnitude as for the *y*-parameters.

## The interatomic distances

The following interatomic distances are obtained:

| | | |
|---|---|---|
| $Sb–F_1 = 2.22 \pm 0.03$ Å | $Cs–2\,F_2 = 2.82 \pm 0.2$ Å | $F_1–F_4 = 2.59$ Å |
| $Sb–F_2 = 2.38 \pm 0.2$ Å | $Cs–2\,F_2 = 2.97$ » | $F_2–F_4 = 2.75$ Å |
| $Sb–F_3 = 2.08 \pm 0.2$ Å | $Cs–2\,F_2 = 3.56$ » | $F_3–F_4 = 2.57$ Å |
| $Sb–F_4 = 2.01 \pm 0.2$ Å | $Cs–2\,F_3 = 2.62$ » | (shortest F–F distances) |
| | $Cs–2\,F_4 = 3.30$ » | |
| Mean value: $Sb–4\,F = 2.2$ Å | $Cs–10\,F = 3.0$ Å | |

The structure is shown in Fig. 1. As will be seen, the antimony atoms form four tetrahedral bonds with bond angles 88°, 90°, 107°, 110°, 115°, and 123°. Two tetrahedra are linked together so that an $Sb_2F_7^-$-group is formed. The Cs atoms are surrounded by ten F atoms, which are situated at the corners of a rather irregular polyhedron.

## Comparison with the structures of $SbF_3$ and $KSb_4F_{13}$

The structure of $SbF_3$ is probably built up of $SbF_3$ molecules, the Sb atom forming three strong bonds (5). $SbF_3$ molecules are also found in $KSb_4F_{13}$

with approximately the same Sb—F distance 2.0 Å as in $SbF_3$ (1). It was suggested in a previous paper that in addition to these three strong bonds the antimony atoms in $KSb_4F_{13}$ form some weaker »secondary» bonds of 3.0 Å, so that in fact an $Sb_4F_{13}^-$ complex can be assumed to exist. With three covalent (probably $p^3$) bonds Sb—F, both the Sb and the F atoms in the $SbF_3$ groups will have the formal charge 0. The amount of ionic bond character in these short bonds will, however, give the Sb atom a positive charge and the F atoms a negative charge. The bonds from one Sb atom to the thirteenth F atom can then be described as weak ionic bonds, and such weak ionic bonds must then also exist between one Sb atom and F atoms belonging to another $SbF_3$ group (see 1). As this thirteenth F atom is the only one which does not form a covalent bond with an antimony atom, it may be expected that its negative charge is higher than those of the other F atoms (the K—F bonds being certainly mainly ionic). Then of these »secondary» ionic bonds, — corresponding to Sb—F distances of about 3 Å — the strongest ones are probably formed with this thirteenth, »central» F atom in the $Sb_4F_{13}^-$ complex.

However, in $CsSb_2F_7$ the bonds must be ofanother type. Judging from the interatomic distances the four Sb—F bonds are of the same kind. The Sb—F distances of 2.2 Å are obviously far longer than those generally met with in tetrahedral complexes of oxygen or fluorine atoms and metal atoms. It seems that in this structure too the bonds are mainly covalent. Probably the bond type involves a resonance between *p* and *d* orbitals with the *s* electron pair inert. The formal charges on the atoms in the $Sb_2F_7^-$ group will be —1 for the Sb atoms, 0 for six of the fluorine atoms and + 1 for the seventh fluorine atom, but the amount of ionic character in the bonds will certainly suffice to make the real charges of the Sb-atoms positive and those of the F atoms negative.

## Other $RSb_2F_7$ compounds

As was shown in a previous paper the $RSb_4F_{13}$ compounds form an isomorphous series, R being K, Rb, Cs, $NH_4$, and Tl. However, the corresponding $RSb_2F_7$ all have different structures. In a subsequent paper a survey of these compounds will be given and of complex antimony (III) fluorides of other formula types.

We are indebted to Professor A. WESTGREN for his kind interest in this work and to the Swedish Natural Science Research Council for financial support.

## SUMMARY

The crystal structure of $CsSb_2F_7$ has been determined. The cell dimension are $a = 19.32$ Å, $b = 6.89$ Å, $c = 5.58$ Å, $\beta = 90°$. The space group is $C_{2h}^6 - C\,2/c$ and the atoms are placed in the following positions:

$(000;\ \frac{1}{2}\frac{1}{2}0)$

4 Cs in 4 $(e)$: $\pm\,(0\,y\,\frac{1}{4})$ $y = 0.230$

8 Sb in 8 $(f)$: $\pm\,(xyz);\ \pm\,(\bar{x}\,y\,\frac{1}{2} - z)$ $x = 0.1694$ $y = -0.239$ $z = 0.283$

4 F in 4 $(d)$: $\frac{1}{4}\frac{1}{4}\frac{1}{2};\ \frac{3}{4}\frac{1}{4}0$

8 F in 8 (*f*): $x = 0.0695$ $y = 0.583$ $z = 0.167$

8 F in 8 (*f*): $x = 0.105$ $y = 0.00$ $z = 0.333$

8 F in 8 (*f*): $x = 0.167$ $y = 0.292$ $z = 0.139$.

The antimony atoms are surrounded by four fluorine atoms situated at the corners of an irregular tetrahedron. Two tetrahedra share one fluorine atom, so that $Sb_2F_7^-$ groups are formed. The mean distance Sb–F is 2.2 Å.

Institute of Inorganic and Physical Chemistry, University of Stockholm, December 1950.

REFERENCES: 1) **Byström, A.** och **Wilhelmi, K.-A.,** Arkiv Kemi *3,* 17 (1950). — 2) **Watanabé, T., Atoji, M.,** and **Okazaki, C.,** Acta Cryst. *3,* 405 (1950). — 3) **Byström, A.,** Arkiv Kemi, Miner. *A 18,* No 23 (1944). — 4) **Byström, A.,** Arkiv Kemi, Miner. *A 25,* No 13 (1947). — 5) **Byström, A.** och **Westgren, A.,** Arkiv Kemi, Miner. *B 17,* No 2 (1943).

*Table 1.* Comparison between observed and calculated F-values. Cu-*K*.

| *h k l* | $\lvert F_{obs} \rvert$ | $F_{calc}$ | *h k l* | $\lvert F_{obs} \rvert$ | $F_{calc}$ | *h k l* | $\lvert F_{obs} \rvert$ | $F_{calc}$ |
|---|---|---|---|---|---|---|---|---|
| 4 0 0 | 40 | −31 | 1 3 0 | – | −41 | 0 6 0 | 280 | −329 |
| 6 0 0 | 380 | 471 | 3 3 0 | 70 | −50 | 2 6 0 | — | 19 |
| 8 0 0 | — | −41 | 5 3 0 | 70 | −122 | 4 6 0 | — | −22 |
| 10 0 0 | — | 41 | 7 3 0 | 80 | −66 | 6 6 0 | 330 | −267 |
| 12 0 0 | 530 | 492 | 9 3 0 | — | 13 | 8 6 0 | — | 54 |
| 14 0 0 | 50 | −69 | 11 3 0 | 50 | −55 | 10 6 0 | — | 17 |
| 16 0 0 | 75 | 83 | 13 3 0 | 70 | −73 | 12 6 0 | 300 | −284 |
| 18 0 0 | 470 | 352 | 15 3 0 | — | −39 | 14 6 0 | — | 13 |
| 20 0 0 | 75 | −44 | 17 3 0 | 120 | −60 | 16 6 0 | — | −67 |
| 22 0 0 | — | 30 | 19 3 0 | — | −19 | 18 6 0 | 210 | −203 |
| 24 0 0 | 320 | 268 | 21 3 0 | — | −9 | 1 7 0 | 170 | −96 |
| 3 1 0 | — | −26 | 23 3 0 | 40 | −75 | 3 7 0 | — | −10 |
| 5 1 0 | — | 0 | 0 4 0 | 310 | 454 | 5 7 0 | 150 | −163 |
| 7 1 0 | 50 | 66 | 2 4 0 | — | −40 | 7 7 0 | 160 | −120 |
| 9 1 0 | 60 | 77 | 4 4 0 | — | −4 | 9 7 0 | — | 0 |
| 11 1 0 | 40 | 56 | 6 4 0 | 340 | 404 | 11 7 0 | 170 | −128 |
| 13 1 0 | — | −27 | 8 4 0 | — | 15 | 13 7 0 | 160 | −79 |
| 15 1 0 | 90 | −40 | 10 4 0 | — | 48 | 0 8 0 | 250 | 231 |
| 17 1 0 | — | 21 | 12 4 0 | 250 | 343 | 2 8 0 | — | −34 |
| 19 1 0 | — | 46 | 14 4 0 | — | −94 | 4 8 0 | — | −13 |
| 21 1 0 | — | 26 | 16 4 0 | — | 43 | 6 8 0 | 250 | 197 |
| 23 1 0 | 40 | 17 | 18 4 0 | 380 | 314 | 23 1 1 | 110 | 50 |
| 0 2 0 | 230 | −526 | 20 4 0 | 50 | −33 | 21 1 1 | 580 | −253 |
| 2 2 0 | 40 | 93 | 1 5 0 | 150 | 152 | 19 1 1 | 230 | −136 |
| 4 2 0 | 45 | −91 | 3 5 0 | — | 17 | 17 1 1 | 180 | 95 |
| 6 2 0 | 290 | −555 | 5 5 0 | 200 | 69 | 15 1 1 | 690 | −320 |
| 8 2 0 | 50 | 27 | 7 5 0 | 160 | 70 | 13 1 1 | 200 | −121 |
| 10 2 0 | — | 0 | 9 5 0 | 50 | 25 | 11 1 1 | 200 | 49 |
| 12 2 0 | 410 | −425 | 11 5 0 | 100 | 114 | 9 1 1 | 500 | −401 |
| 14 2 0 | — | 49 | 13 5 0 | 130 | 77 | 7 1 1 | 170 | −205 |
| 16 2 0 | — | −65 | 15 5 0 | 70 | 16 | 5 1 1 | 40 | 101 |
| 18 2 0 | 460 | −303 | 17 5 0 | 200 | 112 | 3 1 1 | 330 | −498 |
| 20 2 0 | 70 | 51 | 19 5 0 | 160 | 72 | 1 1 1 | 80 | −83 |
| 22 2 0 | 50 | −75 | | | | | | |

Table 1 (cont.)

| $h\,k\,l$ | $\|F_{\text{obs}}\|$ | $F_{\text{calc}}$ | $h\,k\,l$ | $\|F_{\text{obs}}\|$ | $F_{\text{calc}}$ | $h\,k\,l$ | $\|F_{\text{obs}}\|$ | $F_{\text{calc}}$ |
|---|---|---|---|---|---|---|---|---|
| 1 1 $\bar{1}$ | 60 | −40 | 0 0 2 | 270 | −525 | 2 0 $\bar{4}$ | 200 | 195 |
| 3 1 $\bar{1}$ | 330 | 473 | 2 0 $\bar{2}$ | 70 | −144 | 4 0 $\bar{4}$ | 100 | −63 |
| 5 1 $\bar{1}$ | 80 | 159 | 4 0 $\bar{2}$ | — | 108 | 6 0 $\bar{4}$ | 160 | 261 |
| 7 1 $\bar{1}$ | — | −21 | 6 0 $\bar{2}$ | 210 | −399 | 8 0 $\bar{4}$ | 110 | 179 |
| 9 1 $\bar{1}$ | 500 | 448 | 8 0 $\bar{2}$ | — | −5 | 10 0 $\bar{4}$ | 70 | −58 |
| 11 1 $\bar{1}$ | 80 | 20 | 10 0 $\bar{2}$ | — | −56 | 12 0 $\bar{4}$ | 180 | 312 |
| 13 1 $\bar{1}$ | 140 | 10 | 12 0 $\bar{2}$ | 400 | −432 | 14 0 $\bar{4}$ | 210 | 140 |
| 15 1 $\bar{1}$ | 690 | 322 | 14 0 $\bar{2}$ | 190 | −46 | 16 0 $\bar{4}$ | 180 | −74 |
| 17 1 $\bar{1}$ | — | 28 | 16 0 $\bar{2}$ | 110 | 37 | 18 0 $\bar{4}$ | 220 | 203 |
| 19 1 $\bar{1}$ | — | 29 | 18 0 $\bar{2}$ | 440 | −314 | 20 0 $\bar{4}$ | 120 | 110 |
| 21 1 $\bar{1}$ | 580 | 306 | 20 0 $\bar{2}$ | 60 | 14 | 12 0 6 | 110 | −91 |
| 23 1 $\bar{1}$ | — | −8 | 22 0 $\bar{2}$ | 70 | −27 | 10 0 6 | 190 | −268 |
| 22 0 2 | 120 | −113 | 20 0 4 | 120 | −56 | 8 0 6 | 80 | 88 |
| 20 0 2 | 150 | 78 | 18 0 4 | 190 | 173 | 6 0 6 | 130 | −179 |
| 18 0 2 | 380 | −328 | 16 0 4 | 240 | 257 | 4 0 6 | 170 | −282 |
| 16 0 2 | 160 | −161 | 14 0 4 | 140 | −99 | 2 0 6 | 80 | 101 |
| 14 0 2 | 60 | 63 | 12 0 4 | 200 | 175 | 0 0 6 | 180 | −112 |
| 12 0 2 | 220 | −286 | 10 0 4 | 200 | 185 | 2 0 $\bar{6}$ | 250 | −251 |
| 10 0 2 | 50 | −122 | 8 0 4 | 140 | −56 | 4 0 $\bar{6}$ | 100 | 87 |
| 8 0 2 | 60 | 80 | 6 0 4 | 210 | 284 | 6 0 $\bar{6}$ | 130 | −218 |
| 6 0 2 | 210 | −472 | 4 0 4 | 300 | 287 | 8 0 $\bar{6}$ | 150 | −227 |
| 4 0 2 | 80 | −84 | 2 0 4 | 130 | −121 | 10 0 $\bar{6}$ | 60 | 98 |
| 2 0 2 | — | 9 | 0 0 4 | 410 | 279 | 12 0 $\bar{6}$ | 160 | −164 |

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# Higher aliphatic β-keto acids. Preparation and investigation of the crystal behaviour of seventeen homologous normal chain β-keto acids with 8 to 24 carbon atoms

By EINAR STENHAGEN

With 2 figures in the text

The higher normal chain homologues of acetoacetic acid (β-ketobutyric acid) are considered to be intermediates in the metabolism of fatty acids (cf *e.g.* (1)), but in spite of their biological importance very few of them have previously been prepared in a pure state, and their crystal behaviour does not appear to have been studied. The unstable nature of the free β-keto acids and the fact that esters of the higher β-keto acids have previously been somewhat inaccessible are no doubt responsible for this lack of knowledge. The esters have recently become more accessible, however, as HUNSDIECKER (2) has shown that the cleavage of C-acylated acetoacetic esters with sodium methoxide furnishes a convenient route to methyl esters of higher β-keto acids. The method was previously known, cf *e.g.* BOUVEAULT and BONGERT (3). WAHLBERG (4) gives a review of preparative methods and a complete bibliography of papers dealing with β-keto esters up to the year 1914. The synthesis of the methyl esters of the normal chain β-keto acids with 8 to 24 carbon atoms, using a slightly modified BOUVEAULT-BONGERT-HUNSDIECKER procedure, has been described by STÄLLBERG-STENHAGEN (5). These esters were the starting material in the preparation of the β-keto acids described in this communication.

As pointed out by MITZ, AXELROD and HOFMANN (6), alkaline hydrolysis is unsuitable for the preparation of β-keto acids from the corresponding esters, and for the preparation of the four β-keto acids with 14, 16, 18 and 20 carbon atoms respectively, the authors mentioned used acid hydrolysis with hydrochloric acid in glacial acetic acid solution. The method is very satisfactory and has been used in the present work; but, as described in the experimental part, the original procedure requires some modification in the case of acids with more than 20 or less than 11 carbon atoms.

Of the seventeen β-keto acids now prepared, nine have previously been reported in the literature, and the recorded melting points are included in Table I. As the β-keto acids start to decompose into methyl ketone and carbon dioxide immediately on melting, the determination of the exact melting points is difficult. A small amount of impurity will cause decomposition to begin before the true melting point is reached, which explains why BREUSCH and

*Table I*

| Number of carbon atoms in β-keto acid | Melting point (decomposition temperature)° C | | Long crystal spacing Å | Side spacings Å | Long crystal spacing of methyl ketone derived from β-keto acid. Å |
|---|---|---|---|---|---|
| | Present investigation | Literature | | | |
| 8 | 68.5— 69 | 73— 74[1] | 20.9 | 4.48, 4.27, 4.11, 3.94 | |
| 9 | 68 — 68.5 | | 23.5 | 4.50, 4.28, 3.94, 3.70 | |
| 10 | 79.5— 80 | 80— 85[2] | 24.9 | 4.48, 4.19, 3.93, 3.56 | |
| 11 | 78.5— 79 | 72— 75[2] | 27.6 | 4.45, 4.30, 3.92, 3.70 | |
| 12 | 86.5— 87 | | 29.4 | 4.48, 4.25, 3.94, 3.60 | |
| 13 | 85.5— 86 | 58— 65[2] | 31.8 | 4.48, 4.31, 3.90, 3.70 | |
| 14 | 91 — 91.5 | 93— 94[3] | 33.8 | 4.48, 4.25, 3.91, 3.62 | 36.7 |
| 15 | 90 — 90.5 | | 35.8 and 42 | | 39.3 |
| 16 | 95 — 95.5 | 98— 98.5[3] | 37.8 | 4.47, 4.25, 3.90, 3.64 | 41.8 |
| 17 | 95 — 95.5 | | 40.1 | 4.47, 4.31, 3.88, 3.70 | 44.2 |
| 18 | 98 — 99 | 102—103[3] | 42.0 | 4.47, 4.25, 3.88, 3.64 | 46.9 |
| 19 | 97.5— 98 | | 44.3 and 49 | | 49.4 |
| 20 | 100.5—101 | 104—105[3] | 46.2 | 4.45, 4.26, 3.89, 3.67 | 52.0 |
| 21 | 100 —100.5 | | 48.4 | 4.42, 4.31, 3.88, 3.71 | 54.5 |
| 22 | 102.5—103 | | 50.3 | 4.42, 4.26, 3.87, 3.67 | 57.0 |
| 23 | 102.5—103 | | 52.6 and 59 | | 59.5 |
| 24 | 104.5—105 | | 54.6 | 4.42, 4.26, 3.82, 3.67 | 62.2 |

[1] LOCQUIN (8).
[2] BREUSCH and KESKIN (7).
[3] MITZ, AXELROD and HOFMANN (6).

KESKIN (7), who reported small scale preparations of the $C_{10}$—$C_{13}$ β-keto acids by alkaline hydrolysis of the esters, give melting points for the $C_{11}$ and $C_{13}$ members which are lower than those now found (cf Table I). On the other hand, the unstable nature of the acids may incline the experimenter to determine the melting point with too high a rate of heating, which makes the recorded values too high. This is probably the reason why the reported melting point for the $C_8$ acid prepared by LOCQUIN (8) and those for the four acids ($C_{14}$, $C_{16}$, $C_{18}$ and $C_{20}$) prepared by MITZ *et al.* (6) are higher than the values now found. The authors just mentioned have not indicated the rate of heating employed.

MOUREU and DELANGE (9) have prepared the $C_8$, $C_9$ and $C_{12}$ β-keto acids from the corresponding α-β-acetylenic acids. The β-keto acids are reported as crystalline, but no melting point or analyses are recorded. Their ready decomposition into carbon dioxide and methyl ketone is pointed out.

The melting (decomposition) points of the β-keto acids determined in the course of the present work are plotted against the number of carbon atoms in Fig. 1. The figure shows an odd-even alternation of the type found for many homologous series of long chain compounds. The melting points appear to fall on two smooth lines, the line for the even-numbered members lying above that for the odd-numbered members. The melting points recorded in Table I and Fig. 1 have been obtained with a heating rate over the last 10° before the m.p. of approximately one degree per minute. At a slower rate of heating the decomposition will start at a slightly lower temperature. For example, for a

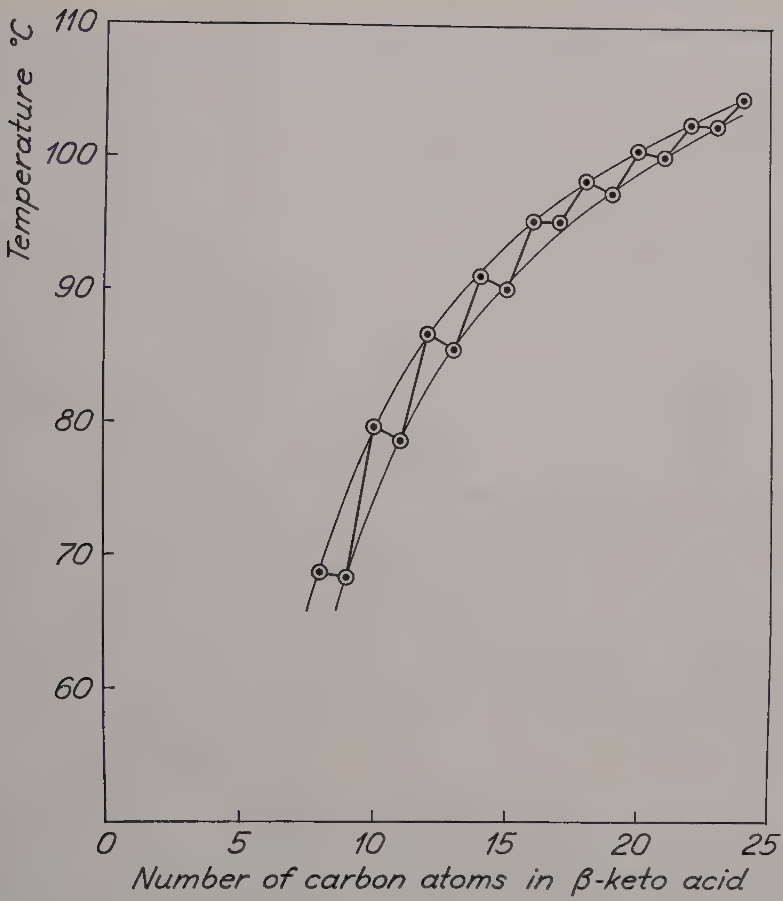

Fig. 1. Melting (decomposition) points of normal chain β-keto acids.

specimen of 3-ketopentadecanoic acid which had been kept in the laboratory for three months, melting with decomposition was found to begin at 89.5° when the rate of heating was 3° per minute. The same result was obtained for a heating rate of 1° per minute, but when the rate of heating was decreased to 1° per 2 minutes, decomposition began at 88.5°. When the specimen was heated from room temperature at a rate of 1° per 6 minutes, decomposition began at 86°.

The lower β-keto acids, which on decomposition form ketones which melt at or below room temperature, undergo a slow decomposition on keeping at the ordinary temperature of the laboratory (cf (9)). The smell of ketone is detectable even in the purest specimens. The higher acids, on the other hand, appear to be fairly stable at room temperature. After they have been kept for several months in the laboratory, melting and decomposition start slightly earlier (0.5 to 1°) than for freshly prepared specimens, but the diffraction patterns of the corresponding methyl ketone has not been observed in the X-ray photographs.

The β-keto acids studied are beautifully crystalline substances. The lowest

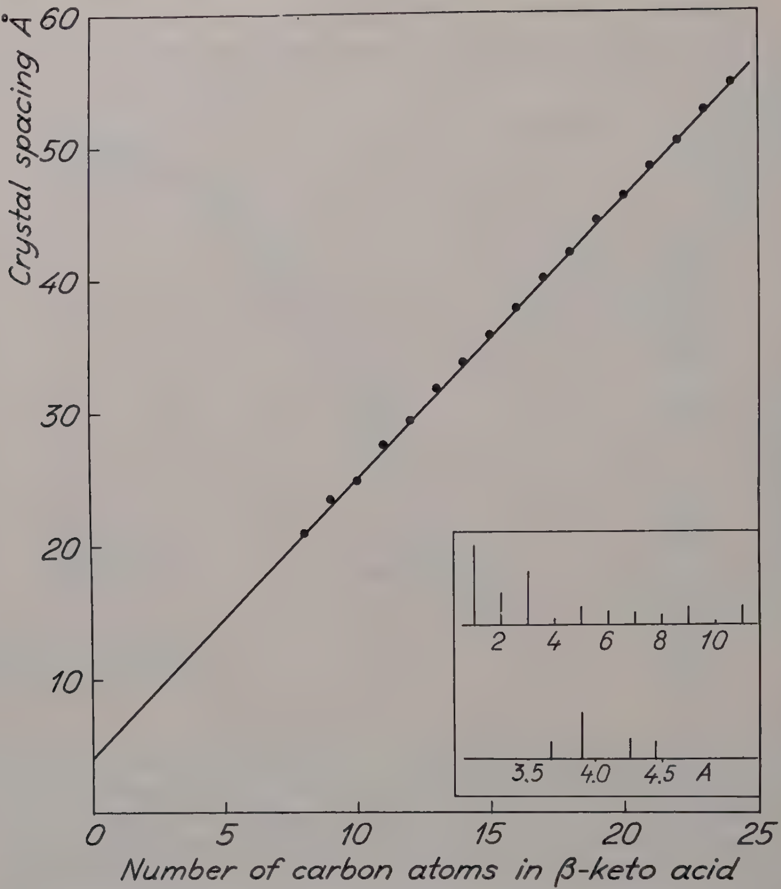

Fig. 2. Long crystal spacings of normal chain β-keto acids. Line drawn through the points of the even-numbered acids. Inset: Visually estimated relative intensities of the first eleven 00*l* reflexions (upper part) and for the four longest side spacings (*hk*0 reflexions) of 3-ketoeicosanoic acid.

members ($C_8$ to $C_{11}$) have been obtained in the form of large, thin, transparent sheets. 3-Ketododecanoic acid is readily obtained from acetone in the form of well-developed single crystals up to 1 cm in length. The size of the crystals obtained under ordinary conditions of crystallization decreases with increasing chain length, and the highest even-numbered members studied ($C_{22}$ and $C_{24}$) crystallize from acetone in the form of small lustrous, lofty plates.

The X-ray data are collected in Table I. No polymorphism has been observed for the even-numbered acids. When plotted against the number of carbon atoms the long crystal spacings of the acids fall on a straight line (Fig. 2), which on extrapolation to zero carbon atoms cuts the ordinate at 4 Å, indicating a layer gap of fairly normal size. With the possible exception of the lowest acid, whose side-spacing pattern differs slightly from those of the higher members, the even-numbered acids appear to form an isomorphous series showing only a very small gradual shift in the side spacings as the chain length is increased. A typical side-spacing pattern is shown in the lower part of the inset of Fig. 2.

The odd-numbered acids also seem to form an isomorphous series, and their long spacings appear to fall on a line lying slightly above that of the even-numbered members. The distance between the lines is of the same order of magnitude as the experimental error, however. Specimens of the $C_{15}$, $C_{19}$ and $C_{23}$ acids crystallized from acetone were found to be mixtures of two different crystalline modifications. With the aid of the recording high temperature camera (10) it was found that the form with the longest spacing (corresponding to the form not isomorphous with that of the other members) disappeared on heating at 48—50°, 59—60° and 68—69° respectively. All acids therefore seem to possess the same crystal structure near the melting point. It is seen from Table I that the longer of the two long spacings for the $C_{19}$ and $C_{23}$ acids is numerically practically identical with that of the corresponding ketone. It should be pointed out that the long spacing of the acid does not arise from the presence of ketone in the specimen, as the intensity variation among the different orders in each case is quite different from that of the ketone. The temperatures at which the diffraction pattern in question disappears (cf above) are also higher than the melting points of the corresponding ketones.

The long crystal spacings of the methyl ketones formed on thermal decomposition of the β-keto acids have been measured for ketones solid at room temperature. The data are included in Table I. Methyl ketones crystallize in the orthorhombic system with vertical double molecules (cf *e.g.* (11, 12) and the spacings for both odd- and even-numbered ketones fall on the same line, which on extrapolation indicates a layer gap of 3.8 Å.

## Experimental

*Preparation of β-keto acids.* The procedure described by Mitz, Axelrod and Hofmann (6) was used for the $C_{11}$ to the $C_{20}$ member of the series. 0.5 G of the methyl ester was dissolved in glacial acetic acid, concentrated hydrochloric acid added dropwise until the solution became slightly turbid, and the mixture left at room temperature for 2 days. The crystals were filtered off and dried *in vacuo* at room temperature. Mitz *et al.* purified the crude β-keto acids by repeated crystallization from acetone, but it was found more convenient first to extract ketone present by means of low-boiling light petroleum (b.p. below 40°), in which solvent the β-keto acids are practically insoluble, and then crystallize the acids from acetone. Only one crystallization was usually needed to obtain the pure acid. The yield of pure material by the above procedure was 50–70 % of the theoretical. As the main interest was to prepare pure compounds, no attempt was made to obtain the highest possible yield in every case.

In one preparation of the $C_{12}$ acid, formic acid was substituted for acetic acid in the hydrolysis. The yield was the same as when acetic acid was used.

In the case of the lower members a shorter hydrolysis time was usually used, as extensive decomposition sometimes began after about one day, indicated by the formation of gas bubbles within the reaction mixture. During the hydrolysis of the ester of the $C_{10}$ acid, crystals were formed after a few hours, and had to be removed soon after formation.

No crystalline precipitate was obtained in the case of the $C_9$ and $C_8$ acids even after 48 hours. After about 24 hours water was therefore added, and the mixture placed in the refrigerator at 0°. The β-keto acid separated in the form of very large, thin, transparent plates. These were washed with low-boiling light petroleum. The β-keto acid was then recrystallized by low temperature crystallization from a mixture of acetone and low-boiling light petroleum, using a cooling bath of solid carbon dioxide and ethanol.

When the hydrolysis procedure of Mitz *et al.* was applied to methyl esters of acids with more than 20 carbon atoms, it was found that the β-keto ester tended to separate on the addition of the hydrochloric acid. This was remedied by adding an amount of benzene corresponding to about 20 % of the glacial acetic acid used. Addition of too much benzene will cause separation into two liquid phases.

The analyses are given in Table II.

*Table II*

Analysis of β-keto acids.

| Systematic name | Per cent composition | | | |
|---|---|---|---|---|
| | C | | H | |
| | Calcd | Found | Calcd | Found |
| 3-Ketooctanoic acid | 60.74 | 61.31 | 8.92 | 9.05 |
| 3-Ketononanoic acid | 62.76 | 62.68 | 9.37 | 9.32 |
| 3-Ketodecanoic acid | 64.49 | 64.66 | 9.74 | 9.74 |
| 3-Ketohendecanoic acid | 65.97 | 66.27 | 10.07 | 9.92 |
| 3-Ketododecanoic acid | 67.25 | 67.26 | 10.35 | 10.41 |
| 3-Ketotridecanoic acid | 68.31 | 68.28 | 10.60 | 10.59 |
| 3-Ketotetradecanoic acid | 69.38 | 69.43 | 10.81 | 10.88 |
| 3-Ketopentadecanoic acid | 70.27 | 70.57 | 11.01 | 11.12 |
| 3-Ketohexadecanoic acid | 71.07 | 70.94 | 11.18 | 11.26 |
| 3-Ketoheptadecanoic acid | 71.78 | 72.34 | 11.34 | 11.30 |
| 3-Ketooctadecanoic acid | 72.43 | 72.88 | 11.48 | 11.65 |
| 3-Ketononadecanoic acid | 73.03 | 73.05 | 11.61 | 11.59 |
| 3-Ketoeicosanoic acid | 73.57 | 73.57 | 11.73 | 11.74 |
| 3-Ketoheneicosanoic acid | 74.06 | 74.34 | 11.84 | 11.97 |
| 3-Ketodocosanoic acid | 74.52 | 74.94 | 11.94 | 11.79 |
| 3-Ketotricosanoic acid | 74.94 | 74.88 | 12.03 | 11.93 |
| 3-Ketotetracosanoic acid | 75.34 | 75.50 | 12.12 | 12.19 |

The techniques used in the X-ray investigations and in the melting point determinations have been described previously, cf *e.g.* (5, 11).

The author is indebted to Mr. L. Ahlquist and Farm. kand. Gunnel Ställberg for assistance in part of the experimental work, to Miss A. Renman of the Microanalytical laboratory for the analyses, and to *Statens Medicinska Forskningsråd* for financial support.

## SUMMARY

Seventeen normal chain β-keto acids with 8 to 24 carbon atoms have been prepared. Melting-point data and crystal spacings are recorded. Polymorphism has only been observed for three of the odd-numbered acids, and all acids seem to adopt the same crystalline form near the melting (decomposition) point.

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REFERENCES. 1. **Breusch, F. L.,** Advances in Enzymol. *8,* 343 (1948). — 2. **Hunsdiecker, H.,** Berichte *75,* 477 (1942). — 3. **Bouveault, L.,** and **Bongert, A.,** Bull. Soc. Chim. (3), *27,* 1088 (1902). — 4. **Wahlberg, E.,** »Om pivalylättikester», Thesis, Uppsala 1914. — 5. **Ställberg-Stenhagen, S.,** Arkiv för Kemi, Min. & Geol. *20 A,* No. 19 (1945). — 6. **Mitz, M. A., Axelrod, A. E.,** and **Hofmann, K.,** J. Amer. Chem. Soc. *72,* 1231 (1950). — 7. **Breusch, F. L.,** and **Keskin, H.,** Rev. faculté sci. univ. Istanbul *11 A,* 24 (1946). — 8. **Locquin, R.,** Bull. Soc. Chim. [3], *31,* 595 (1904). — 9. **Moureu, C.,** and **Delange, R.,** Bull. Soc. Chim. (3), *29,* 667 (1903). — 10. **Stenhagen, E.,** Acta Chem. Scand., in the press. — 11. **Saville, W.,** and **Shearer, G.,** J. Chem. Soc. *1925,* 591. — 12. **Oldham, J. W. H.,** and **Ubbelohde, A. R.,** Trans. Faraday Soc. *35,* 328 (1939).

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# Beziehungen zwischen dem Alter und dem Wirkstoff-Gehalt normaler und canceröser Organe. I

Von Hans von Euler

Organe, besonders endokrine Drüsen *normaler* Tiere zeigen in hohem Alter nicht selten solche qualitativen oder quantitativen Veränderungen des Stoffwechsels, welche bei *cancerösen* Tieren schon in einer früheren Lebensperiode eintreten. Vergleichende Beobachtungen dieser Art sind zuerst wohl bezüglich der Hypophyse gemacht worden, ferner bezüglich der Schilddrüse, welche, wie die Nebennieren, mit zunehmendem Alter Rückbildungen unterliegt und atrophisch wird.

Die Alters-Degenerationen der einzelnen Organe und die Vorgänge, durch welche dieselben sich im übrigen Organismus auswirken, sind in chemischer Hinsicht noch wenig erforscht und sollen hier nicht näher besprochen werden. Der experimentellen Erforschung zugänglich sind bis jetzt nur die quantitativen Veränderungen einiger Sekretstoffe der endokrinen Drüsen, zu denen wir sowohl die specifischen Hormone wie auch die Inhibitoren und Wachstumsstoffe der Enzymsysteme zählen.

Bei der Beurteilung der Abhängigkeit der Wirkstoffmengen einerseits vom Lebensalter, andererseits vom cancerösen Zustand der Tierorgane, stösst man auf die principiell wichtige Frage, ob der Organismus altert und sich dem Tode nähert, wenn die Wirkstoffe in den einzelnen Organen bezw. in einem besonders schwachen Organ ihre normale Menge und Aktivität geändert haben, oder ob umgekehrt diese Änderungen die *Folgen* davon sind, dass der Organismus seine Lebensfähigkeit verloren hat. Eine Entscheidung, welches die primären Ursachen und welches die Folgen sind lässt sich noch nicht treffen, und wir können uns also auf die nachstehenden Überlegungen beschränken:

Bekanntlich hat Carrel[1] auf Grund seiner Beobachtungen an Gewebskulturen die Zelle für unsterblich erklärt, weil bei Jahre lang fortgesetzter Züchtung die Zellteilung nicht zum Stillstand kommt. In einer eingehenden Kritik der Folgerungen, welche Carrel aus seinen Versuchen zog, hat Albert Fischer[2] hervorgehoben, dass die Gewebskulturen ihre Wachstumsfähigkeit d. h. ihr Leben aufrechterhalten *sofern* sie durch häufige Erneuerung des Mediums mit Wirkstoffen versorgt bleiben und von Hemmungsstoffen befreit werden.

„The following conclusions must be drawn: The inherent growth energy of a tissue at a certain time, both in vitro and in vivo, is a function of its earlier activity and of the concentration of growth-promoting and growth-inhibiting substances in the surrounding medium.“

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[1] A. Carrel, Man the Unknown. (1935).

[2] Albert Fischer, Biology of Tissue Cells. Kopenhagen, Gyldendal 1946.

Zur Beantwortung der Frage: Wodurch altert der tierische Organismus? besitzen wir zwei Ausgangspunkte:

1. Mangel an Wachstums-Stoffen,
2. Auftreten von Hemmungsstoffen (Inhibitoren), die im gealterten Organismus nicht mehr rasch und vollständig genug aus den Zellen entfernt werden.

Hierzu ist zu bemerken, dass der Begriff „Wuchs-Stoff“ oft zu eng gefasst worden ist. Zu den Wuchsstoffen sind zu rechnen alle diejenigen Stoffe, welche in kleinen Mengen als Katalysatoren die Entwicklung von Organismen fördern; also in erster Linie die Co-Enzyme bezw. die katheptischen Gruppen lebenswichtiger Enzyme, wie u. a. die Niacin-Adenin-Nukleotide (Cozymase und Codehydrase II), Flavin-Adenin-nukleotide (als prosthetische Gruppen der Xanthinoxydase, der Diaphorase der Aminosäuren-Oxydase), ferner die Eisenporphyrine (Cytochrom und Cytochrom-Derivate KEILINS), die Cu-haltigen prosthetischen Gruppen der Polyphenoloxydasen, die Zn-haltige Gruppe der $CO_2$-Anhydratase.

Wichtige Wuchsstoffe sind ferner die Vitamine, die ihre aktivierenden Wirkungen in Verbindung mit specifischen Proteinen (Apo-Enzymen) ausüben. Dies gilt nicht nur vom fettlöslichen Vitamin A, das schon von seinem Entdecker Fr. G. HOPKINS durch seine Wirkung auf junge Ratten gekennzeichnet wurde, sondern in ebenso hohem Grad von den wasserlöslichen Vitaminen der B-Gruppe einschliesslich der Folsäure und nicht weniger vom Vitamin C, der Ascorbinsäure. Als wichtigste Klasse unter den Wuchsstoffen sind schliesslich die Hormone zu nennen, die Produkte endokriner Drüsen, deren Wirkungen ebenso tiefgreifend wie vielseitig sind, und welche teils als Synergisten, teils als Antagonisten in die Funktionen der Vitamine und anderer Wuchsstoffe eingreifen.

Alle diese Wuchsstoffe sind, wie erwähnt, mehr oder weniger fest an Proteine gebunden und sind dementsprechend mehr oder weniger leicht in freiem Zustand isolierbar. Mehrere von ihnen werden von höheren Tieren synthetisiert; eine wesentliche Ausnahme macht bekanntlich die Ascorbinsäure, welche dem Menschen, dem Meerschweinchen und wenigen anderen Tieren zur Deckung ihres Bedarfs mit der Nahrung zugeführt werden muss.

In der vorliegenden Mitteilung soll besprochen werden, wie sich die Bildung und Aktivität der Ascorbinsäure mit dem Alter und dem sonstigen physiologischen Zustand von Versuchstieren und — soweit bekannt, beim Menschen, ändert.

### Ascorbinsäure

Das Vitamin C wird bekanntlich im Organismus nicht, wie z. B. Vitamin A, gespeichert, sondern wird bei überschüssiger Aufnahme, sei es per os, sei es durch Injektion, innerhalb weniger Stunden durch den Harn abgegeben. Es findet sich im Organismus in zwei Oxydations-Stadien, der Ascorbinsäure und der Dehydro-ascorbinsäure, welche ein reversibles Gleichgewicht bilden und leicht ineinander übergehen. Demgemäss ist Dehydroascorbinsäure antiscorbutisch etwa ebenso wirksam wie Ascorbinsäure selbst.

Durch die gebräuchlichste Methode der chemischen Ascorbinsäure-Bestimmung, nämlich mit Tillmans-Reagens, Dichlorphenol-Indophenol, wird nur die Ascorbinsäure erfasst; soll auch die Dehydroascorbinsäure gemessen werden, so muss man dieselbe zuerst in geeigneter Weise, meist durch $H_2S$, reduzieren.

Mit den Ergebnissen der Titrationsanalyse durch Dichlorphenol-Indophenol gehen die Ergebnisse der biologischen Prüfung auf antiscorbutische Wirkung an Meerschweinchen parallel. Die Rolle der Ascorbinsäure als C-Vitamin beschränkt sich aber keineswegs auf ihren antiscorbutischen Effekt, vielmehr ruft der Mangel an Ascorbinsäure mannigfache Störungen im Organismus hervor, welche nicht als Symptome des Skorbuts angesehen werden können.

Welche chemischen Vorgänge die einzelnen Symptome veranlassen, ist unbekannt, und deswegen wissen wir auch nicht, durch welche Reaktionen die Ascorbinsäure an den einzelnen Reaktionen beteiligt ist.

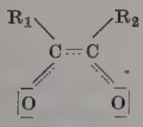
Ascorbinsäure gehört zur Körperklasse der Reduktone[1], ihre Konstitution wurde zusammen mit derjenigen des Triose-Reduktons von EULER und MARTIUS[2] aufgeklärt, während gleichzeitig HAWORTH[3] und seine Schule von anderen Ausgangspunkten her Beweise für diese Konstitution beibrachten. Man darf annehmen, dass die mannigfachen Wirkungen der Ascorbinsäure auf ihrer Endiolgruppe beruhen, und dass ihre Rolle als Oxydo-Reduktions-Katalysator mit ihrem Redox-Potential eng verknüpft ist.

## Oxydation der Ascorbinsäure

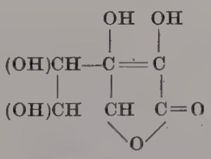
Die Reaktionsbedingungen, unter welchen Ascorbinsäure der Oxydation durch Dichlorphenol-indophenol (Tillmans-Reagens, TR) und durch andere Farbstoffe unterliegt, sind in unserer Monographie angegeben und brauchen hier nicht näher beschrieben zu werden. Das Oxydationsprodukt, die Dehydro-ascorbinsäure, entsteht reversibel, und zwar sowohl in alkalischer wie auch in saurer Lösung. Das Redox-Potential wurde potentiometrisch ($E_0$) von BALL[4], polarographisch $(\pi_{1/2})_0$ von BRDIČKA[5] gemessen:

$$E_0 = 0{,}390 \text{ Volt}$$
$$(\pi_{1/2})_0 = 0{,}595 \text{ Volt}$$

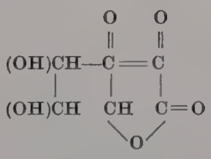
Die Oxydation der Endiol-Hydroxyle führt zur Bildung der Diketogruppe. Sie verläuft nach unserer Annahme (EULER, Chimia, 3 209; 1949) bei Verwendung von Tillmans-Reagens und anderer Farbstoffe in der Weise, dass der oxydierende Farbstoff, der selbst reduziert wird, dem doppelt gebundenen C-Atompaar 2 $\pi$-Elektronen entzieht, wodurch das Biradial (II) entsteht.



(II)



Ascorbinsäure



Dehydroascorbinsäure

Unter anderen Reduktionsindikatoren seien hier noch erwähnt:

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[1] Vgl. EULER u. H. HASSELQUIST, Reduktone. Stuttgart, Enke, 1950.

[2] EULER u. C. MARTIUS, Sv. Kem. Tidskr. 45, 73 (1933). — Lieb. Ann. 505 73, (1933).

[3] W. N. HAWORTH, Hirst, Percival a. F. Smith, J. Chem. Soc. 1270 (1933).

[4] G. BALL, J. Biol. Chem, 118, 219 (1937).

[5] Vgl. Monographie, S. 38.

a) o-Dinitrobenzol und o-Dinitrobenzoesäure, eingeführt von Kuhn und Weygand[1], dann von Fearon und Kaweran[2], besonders verwendet in den Arbeiten von O'Meara[3] und von Cocker[4],

b) das Triphenyltetrazoliumchlorid[5].

Die Rückoxydation des reduzierten Tillmans-Reagenses (des Leuko-Dichlorphenol-Indophenols) lässt sich mit Chinon, Chinhydron, m-Dinitrobenzol u. a. durchführen: vgl. dieses Arkiv 1, Nr 30; 1949.

Die Oxydation der Ascorbinsäure durch Sauerstoff wird schon durch minimale Mengen von Cu-Salzen katalysiert. Die ersten diesbezüglichen Versuche von Euler, Myrbäck und Larsson[6] wurden bald darauf von Kellie und Zilva[7] bestätigt und erweitert. Die Oxydation geht dabei z. T. über das Stadium der Dehydroascorbinsäure hinaus und wird irreversibel. Diese Cu-Katalyse wird (in vitro) durch Zusätze von Organ-Extrakten gehemmt. Ein weiterer, bemerkenswerter Katalysator der Ascorbinsäure-Oxydation durch Sauerstoff ist die Gallussäure (Zilva, Biochem. J. 36, 64 (1942).

Wie zu erwarten war, ist die Geschwindigkeit der Oxydation durch Sauerstoff der anwesenden Cu-Menge nicht proportional, sondern nimmt langsamer zu als diese. Ein Vergleich der Sauerstoffaufnahme durch äquimolekulare Konzentrationen von Ascorbinsäure und den beiden Triose-Reduktonen ergab Parallelität zwischen Ascorbinsäure und Transredukton,[8] [9] wonach ev. auch die Transform für die l-Ascorbinsäure in Betracht gezogen werden kann.

Peroxydase oxydiert u. a. Ascorbinsäure, Dioxymaleinsäure, Polyphenole sowie Fe$^{II}$-Cytochrom[10].

## Ascorbinsäure im Stoffwechsel der Nebennieren

Ascorbinsäure als Redox-Katalysator ist am Stoffwechsel der Nebennieren wesentlich beteiligt, indem sie als Oxydations-Schutz des Adrenalins fungiert.[11] Damit ist aber die Bedeutung der Ascorbinsäure für die Nebennieren keineswegs erschöpfend beschrieben. In erster Linie ist an die Rolle der Ascorbinsäure als

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[1] R. Kuhn und F. Weygand, Ber. 69, 2969 (1936).

[2] Fearon u. Kaweran, Biochem. J. 37, 326 (1943).

[3] R. A. Q. O'Meara et al. Biochem J. 45, 24 (1949).

[4] W. Cocker, R. A. Q. O'Meara et al. Soc. 1950, 2052.

[5] Siehe hierzu I. Hausser, D. Jerchel u. R. Kuhn, Ber. 82, 195 (1949).

[6] Euler, K. Myrbäck und H. Larsson, Z. physiol. Chem. 217, 1 (1933).

[7] A. E. Kellie u. S. S. Zilva, Biochem. J. 29, 1028 (1935).

[8] Euler u. H. Hasselquist, Z. physiol. Chem. im Druck. — Eine besondere Rolle kommt den Cu-Fe-Porphyrinen zu.

[9] F. Arndt, L. Loewe u. E. Ayca, Ber. 84, 333, haben interessante vergleichende Versuche über die Geschwindigkeit der Oxydation von Endiolen durch $Fe^{+++}$ angestellt. Die dafür erforderliche Zeit in 0,001 n. alkohol. Lösung mit $^1/_4$ Aequiv. TR und 1 Aequiv. $Fe^{+++}$ steigt in der Reihenfolge: Ascorbinsäure, Triose-Redukton (Redukton A), Thiacumarindiol und Carbostyrildiol.

[10] Britton Chance, Science 109, 204 (1949).

[11] Euler u. E. Klussmann (Dieses Arkiv 11 B Nr 7; 1933) haben bereits 1932–33 den Einfluss von Adrenalin-Injektionen auf den Ascorbinsäure-Gehalt der Nebennieren untersucht. Da im Nebennierenmark das Adrenalin entsteht, hat die Ascorbinsäure auf diesem Weg auch einen (indirekten) Einfluss auf einen wichtigen Teil des Nervensystems. Andererseits werden die *psychischen* Einflüsse auf die Sekretion der Nebennieren durch die Vermittlung des Adrenalins verständlich.

Wasserstofr-überträger zu erinnern. Vermutlich wird durch die Ascorbinsäure das Ringsystem von Doca oxydativ angegriffen.

Wie sich durch Arbeiten von SELYE[1], RINEHART[2], SCHAFFENBURG[3] u. a. gezeigt hat, besteht eine enge Zusammenwirkung der Ascorbinsäure mit den steroiden Hormonen der Nebennierenrinde, besonders mit dem Cortison und dem Desoxycorticosteron. Demgemäss nimmt bei Injektion von Desoxycorticosteron-acetat (Doca) die titrierbare Menge der Ascorbinsäure in den Nebennieren der Meerschweinchen erheblich ab. Die Versuche der genannten Forscher haben grosses medicinisches und biochemisches Interesse erweckt, weil nachgewiesen werden konnte, dass eine gewisse Ähnlichkeit besteht zwischen den experimentell durch Doca hervorgerufenen Schäden und den bei rheumatischem Fieber beobachteten Symptomen. PEMBERTON et al.[4] konnten durch Injektion von Doca in etwa der Hälfte der Versuchstiere experimentelle Arthritis hervorrufen.

Was nun den *Einfluss des Alters* auf den Stoffwechsel der Nebennieren betrifft, so ist seit langer Zeit bekannt, dass mit zunehmendem Alter die Nebennierenrinde atrophiert, und zwar ist diese Atrophie viel ausgeprägter als die der Marksubstanz. Durch Eingabe von Doca haben SELYE und DOSNE[5] die Atrophie hervorrufen können.

Man konnte vermuten, dass der Ascorbinsäure-Gehalt der Nebennieren mit zunehmendem Alter abnimmt, und wir haben deshalb diesbezügliche Versuche an Ratten vom mittleren Gewicht 300 g angestellt. Der Ascorbinsäure-Gehalt der frischen Nebennieren betrug im Mittel 3,1 $\gamma$/mg frische Nebenniere.

Frühere Bestimmungen (Sv. Kem. Tidskr. 47, 27, 1935) hatten ergeben: Mittel 2,2 $\gamma$/mg frische Nebenniere.

Wir geben ein Beispiel ausführlicher an:

*Tabelle 1.*

A. Ratte E 22/2. Gew. 130 g. 8 Wochen alt. 2 Nebennieren vom Totalgew. 20,9 mg
B. Ratte $K_{b2}$ Gew. 280 g. 33 Wochen alt. 2 Nebennieren vom Totalgew. 52,3 mg
C. Ratte M. Mb 1935. Gew. 210 g.

| Ratte | 1 Nebenniere mittl. Gew. | $\gamma$ Ascorbins. | $\gamma$ Ascorbins. per mg fr. Nebenniere |
|---|---|---|---|
| A | 10,45 | 37,65 | 3,61 |
| B | 26,15 | 84,00 | 3,21 |
| C | 20,11 | | 3,20 |

Eine stärkere Abnahme des Ascorbinsäure-Gehaltes setzt vermutlich erst bei noch höherem Alter ein.

Auch in Tumor-tragenden Ratten (Walker 256 Carcino-Sarkom) erweist sich der Ascorbinsäure-Gehalt der Nebennieren nicht wesentlich kleiner als derjenige der gleichalteriger normaler Ratten. Im folgenden als Beispiel angeführten Ver-

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[1] H. SELYE u. Mitarb. J. Am. Med. Ass. 124, I 201.
[2] J. F. RINEHART u. Mitarb. Ann. Rheum. Dis. 3, 154 (1943).
[3] C. SCHAFFENBURG, G. M. C. MASSON u. A. C. CORCORAN, Proc. Soc. exp. Biol. a. Med. 84, 358 (1950).
[4] PEMBERTON, J. Lab. a. Clin. Med. 32, 1127 (1947).
[5] H. SELYE u. DOSNE, Proc. Soc. exp. Biol. a. Med. 44, 65 (1940).

*Tabelle 2.* Vallentuna-Stamm.

D. 2 Normal-Ratten. 4 Nebennieren vom Totalgew. 89,8 mg;
E. 2 mit Walker-Tumoren geimpfte Ratten. 4 Nebennieren vom Totalgew. 91,2 mg;
F. 1 mit Walker-Tumor geimpfte Ratte Nr. 145. 2 Nebennieren vom Totalgew. 55 mg.

| Ratte, 11 Wochen alt | 1 Nebenniere mittl. Gew. | $\gamma$ Ascorbins. | $\gamma$ Ascorbins. per mg fr. Nebenniere |
|---|---|---|---|
| D. Normal | 22,45 | 63,5 | 2,83 |
| E. Walker-Tumor retrograd | 22,80 | 60,5 | 2,66 |
| F. » Nr 145 42 g | 27,5 | 82,00 | 2,98 |

such hatten sämtliche Ratten während 14 Tagen Injektionen von 30 mg Ascorbinsäure erhalten.

Das Altern der Organe ist im allgemeinen von Wasserverarmung begleitet, und dies gilt auch für die Nebennieren. Berechnet man ihren Ascorbinsäure-Gehalt per mg Trockengewicht statt Frischgewicht, so findet man eine Abnahme des Ascorbinsäure-Gehaltes mit steigendem Alter.

Dass Ascorbinsäure als Wasserstoffüberträger wesentlich an dem oxydo-reduktiven Stoffwechsel der Organe beteiligt ist, wurde bereits erwähnt. Diese Funktion wird besonders wichtig in solchen Organen, in welchen Cozymase als Wasserstoffüberträger gleichzeitig mit den zentralen Oxydationssystemen:

Dehydrase-Diaphorase-Cytochrom-Cytochromoxydase ganz zurücktritt, wie besonders in der Augenlinse.[1] Hier ist die Ascorbinsäure Vermittler der Atmung und hier macht sich die mit dem Alter zunehmende Ascorbinsäure-Verarmung besonders bemerkbar: Von dem hohen Ascorbinsäure-Gehalt der jugendlichen Augenlinse[2] sinkt beim Katarakt der Ascorbinsäure-Gehalt auf einen ganz niedrigen Wert herab.[3]

Keineswegs nimmt die Wirkung *aller* Enzymsysteme mit steigendem Alter ab. Es sei nur erwähnt, dass z. B. die Katalase-Wirkung der Leber von Ratten in einer langen Lebensperiode mit steigendem Alter ansteigt.[4] Es muss aber hier die allgemeine Bemerkung eingeschaltet werden, dass die biochemischen Alterserscheinungen in der Regel keineswegs linear vom Reifebeginn bis zum Tod zunehmen, sondern dass der Abfall der Aktivität oft erst in hohem Lebensalter deutlich wird.

## Einfluss der Ascorbinsäure auf die Bildung vitalfärbbarer roter Blutzellen

Auch diese Funktion der Ascorbinsäure ist hinsichtlich des ihr zugrundeliegenden Reaktionsmechanismus nicht aufgeklärt. In einer Reihe von Arbeiten haben EULER und MALMBERG[5] untersucht, welche stofflichen Zusätze die Wirkung der Ascorbinsäure beeinflussen. Bereits in der II. Mitt. wurde auf die Rolle hingewiesen, welche der Ascorbinsäure als Wasserstoffüberträger zukommt.

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[1] Siehe EULER u. C. MARTIUS, Z. physiol. Chem. 222, 65 (1933).

[2] BETH u. HANS V. EULER, Dieses Arkiv 11 Nr. 18 (1933). — BIRCH u. DANN, Nature 131 (1933).

[3] EULER u. G. GÜNTHER, Z. physiol. Chem. 256, 229 (1938).

[4] EULER u. A. GLASER, Deutsche Med. Wochenschr. 75, 631 (1950).

[5] EULER u. M. MALMBERG, Z. physiol. Chem. 243, 121 (1935). — 249, 85 (1937). — 252, 24 (1938). — 256, 243 (1938–39). — 261, 103 (1939). — Naturwissensch. 27, 166 (1939). — Siehe auch SEYDERHELM u. GREBE, Vitamin und Blut. Leipzig, Barth, 1935.

Wie durch KARRER, EULER and HELLSTRÖM[1] festgestellt wurde, tritt bei Zusatz von Hämin in reinem Pyridin zu einer 1 %igen Lösung von Ascorbinsäure eine Farbenveränderung ein, wie sie für Hämochromogen charakteristisch ist. Durch diese glatt verlaufende Reduktion entsteht neben Hämochromogen „grünes Hämin". Nach EDLBACHER und v. SEGESSER[2] entsteht „grünes Hämin", wenn Ascorbinsäure mit hämolysierten Erythrozyten bei pH = 7,2 in Gegenwart von Sauerstoff reagiert.

In der III. Mitt. (l.c. 256, 251 und 261, 103) konnte gezeigt werden, dass die Reticulozythen-Bildung und Ausschüttung auch sehr stark vom Vitamin-A-Zugang bezw. vom A-vitaminotischen Zustand des Tieres beeinflusst wird. Hier liegt eine der zahlreichen Erscheinungen vor, welche auf dem Synergismus der Vitamine C und A beruhen. Auf die Abnahme der vitalfärbbaren roten Blutzellen nach Doca-Injektionen werden Verf. und Dr. NORA BARGONI (vgl. dieses Arkiv 1. 17; 1949) eingehender zurückkommen.

Die Untersuchung wurde mit Unterstützung von *Lotten Bohmans Fond* ausgeführt.

Stockholm, Universität, Vitamin-Institut.

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[1] P. KARRER, EULER u. H. HELLSTRÖM, Dieses Arkiv 11 B Nr 6 (1933). — Vgl. auch LEMBERG, CORTIS-JONES u. NORRIE, Biochem. J. 32, 149 (1938).

[2] EDLBACHER u. v. SEGESSER, Naturwissensch. 25, 461, 557 (1937).

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# Mercaptoacetone and related compounds. A study on some discrepancies in the literature

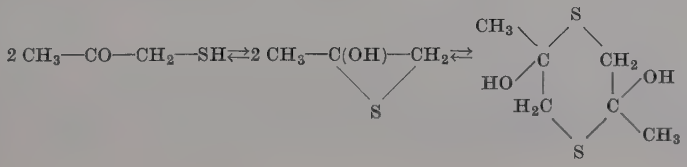
By LENNART SCHOTTE

With 1 figure in the text

In 1926 GROTH (1) prepared the first known aliphatic α-ketomercaptan, namely mercaptoacetone. Monochloro-acetone was treated with dithiocarboxy-glycolic acid — obtained from carbon disulfide and glycolic acid in an alkaline medium — and the resulting product, thiocarb-acetonylmercapto-glycolic acid (I) was decomposed in a weakly acid water solution at 80—82°, the mercaptan being removed by steam at a reduced pressure of about 300 mm. The substance was crystalline and soluble in hot organic solvents. A melting point of 80—82° was given.

However, in 1946 HROMATKA and ENGEL (2) independently investigated mercaptoacetone and they then thought the substance to be the first simple aliphatic α-ketomercaptan in the literature. Monochloro-acetone was added dropwise with cooling and stirring to a water solution of sodium sulfhydrate. The mercaptan was formed during the addition and could easily be removed as a precipitate. Here a melting point of 109—111° for the purest substance was obtained.

The marked discrepancy in the melting points and in other characteristics made any certain conclusions impossible and this investigation was started in order to compare the different results. The two substances were prepared according to the descriptions and both of them could be recrystallized from benzene. Melting points of 81—83° and 107—108° respectively were found in good agreement with the statements. The analyses and equivalent weight determinations by iodine titrations gave supporting values, and determinations of molecular weights by measurements of freezing point lowering in bromoform showed mercaptoacetone to be dimeric. This was already suggested by GROTH, although not quantatively proved, and he pointed out that the substance is dissociated in most solutions, especially upon heating, and states the following equilibrium:



However, most derivatives are related to the monomeric keto form, but this can easily be explained from the formula above. Compounds of dimeric form have also been prepared by GROTH. HROMATKA and ENGEL on the other hand assume the substance to be monomeric from solubility data but no determination was performed, due to its attributed instability. Subsequent investigation has indicated this reasoning questionable.

It is now obvious that the discrepancy in the melting points must be due to sterical or polymorphic causes. A closer study of the formula of the dimeric mercaptoacetone shows the possibility of cis-trans-isomerism (II and III). This could perhaps explain the different forms of the substance. However, it seems more probable from the experiments that polymorphic effects are responsible. When a mixture of equal parts of the two substances is recrystallized from benzene, 80 % of the total amount is regained, and this product shows a melting point of 101—103°. A second crystallization gives the pure higher melting substance. An attempt to get the lower melting form from the other form by simple inoculation of an almost saturated solution failed. However, a transition was successful, when the substance, prepared by the method of HROMATKA and ENGEL, was distilled in steam at reduced pressure. In this manner a substance with the melting point 82–84° was obtained. A recrystallization elevated the melting point about 7°, but this can be easily explained from the suggestion that traces of the higher melting form were left and then gave rise to the observed elevation. Another way to get a higher melting point, from the lower melting form of the mercaptan, is a careful powdering, which treatment elevates the melting point to 93–95°. After this substance was kept for five hours at 50° a melting point of 96–97° was observed, but a further elevation by repetition could not be obtained. According to the last mentioned behaviour, powder photographs can be expected to be almost identical, which is in fact the case (Fig. 1).

The possibility of cis-trans-isomerism cannot be overlooked, however, and in such a case the transition can be explained by the possibility of an equilibrium, in solution between the different dimeric forms and the same monomer, which on cooling shifts to favour formation of the more stable dimer, the higher melting one. However, the effect of powdering and the similarities of the powder photographs make the assumption about different crystalline forms of the substance more probable. Since steam distillation at reduced pressure is essential for the preparation of the lower melting form, it is obvious, why HROMATKA and ENGEL did not obtain it.

The reaction behaviour of the two forms of mercaptoacetone has been carefully compared. Derivatives described by HROMATKA and ENGEL of their form of the substance have thus been prepared from the lower melting form as well and *vice versa*. The agreement between the reactions is complete. Complementary molecular weight determinations of p-nitrobenzoyl-mercapto-acetone have been performed. However, the product obtained in pyridine solution from hydroxylamine hydrochloride and mercaptoacetone is not mercaptoacetoxime, as stated by HROMATKA and ENGEL, but the dioxime of the diacetonyl-disulfide (IV). This is obvious from the failure of the typical mercapto group reactions, RHEINBOLDT's reaction (3) and the reaction with iodine, and from molecular weight determinations. The same substance has also been prepared by GROTH in another way and the products are identical. Another proof of the normal

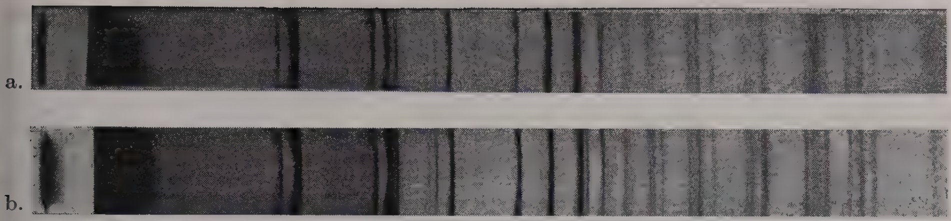


Fig. 1.

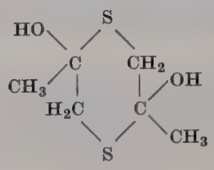
a. Mercaptoacetone (m.p. 81–83° before powdering).
b. Mercaptoacetone (m.p. 107–108°).

monomeric structure of mercaptoacetone in most derivatives, *i.e.* the keto-mercapto form, has been found in the acetonylmercapto-acetic acid (V), prepared in reaction with monobromo-acetic acid. The carboxylic acid could also be obtained from monochloro-acetone and thioglycolic acid. The mixture showed no melting point depression. It can be further mentioned that mercaptoacetone gives a brown nickel precipitate in alcoholic solution, which is soluble in benzene (4). Finally, in point of the dioxime of diacetonylsulfide it may be mentioned that a monohydrate with the melting point 71° can appear as indicated by GROTH (1), while the waterfree oxime has a melting point of 92–94° (1 and 2).

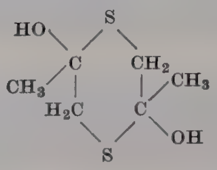
It is of a special interest to discuss more completely the derivative of mercaptoacetone, that is obtained, when one mole of water is removed from the dimeric mercaptan. This compound has been prepared independently by GROTH (1) and HROMATKA and ENGEL (5). However, differing formulae have been proposed by the authors. GROTH, who obtained the substance from mercaptoacetone treated with dilute hydrochloric acid, has given a formula (VI) with two three-membered rings, containing one sulfur atom each. HROMATKA and ENGEL on the other hand prepared the substance by heating the mercaptan on water bath for three hours, during which time the crystals were decomposed. They discussed thoroughly the possibilities of three formulae (VII, VIII and IX) and concluded the structure to be that of a dithiane with an oxygen bridge between two carbon atoms, adjacent to the sulfur atoms (VII). However, it is possible to assume other formulae for the compound. BAKER and BARKENBUS (6) have prepared some dithienes from the corresponding ketomercaptans, $R{-}CO{-}CH_2{-}SH$, by removing two moles of water from the dimeric forms, but when R is a methyl group they state as a result of preliminary work that another kind of reaction is taking place, similar to that giving mesityl oxide and phorone from acetone. This would give rise to a formula (X) with two mercapto groups. This possibility has been discussed by HROMATKA and ENGEL too. Another structure for a dimercaptan (XI) may be imagined as well as one containing a disulfide link (XII).

The substance in question is a colourless oil, which on cooling solidifies to a crystalline mass, melting at 7–8°. It is insoluble in water and sodium hydroxide but soluble and stable in most of the ordinary organic solvents. A partial hydrolysis is observed in acid medium and mercaptoacetone is regained. This can be easily proved by oxidation to the disulfide with iodine, the disulfide being characterized as the dioxime (1). The hydrolysis thus goes almost to completion as the mercaptan is removed from equilibrium by oxidation. The

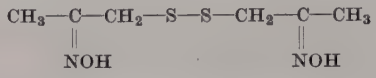
$CH_3—CO—CH_2—S—CS—O—CH_2—COOH$ (I)



(II)



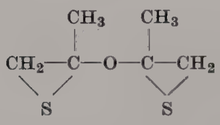
(III)



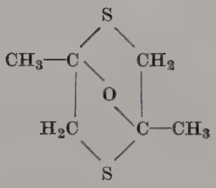
$$CH_3-\underset{\underset{\displaystyle NOH}{\|}}{C}-CH_2-S-S-CH_2-\underset{\underset{\displaystyle NOH}{\|}}{C}-CH_3$$

(IV)

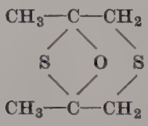
$CH_3—CO—CH_2—S—CH_2—COOH$ (V)



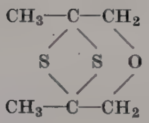
(VI)



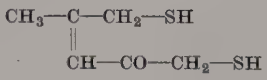
(VII)



(VIII)

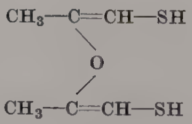


(IX)

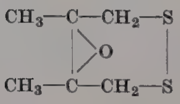


$$\begin{array}{l} CH_3-C-CH_2-SH \\ \qquad\ \| \\ \qquad\ CH-CO-CH_2-SH \end{array}$$

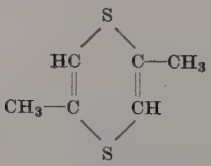
(X)



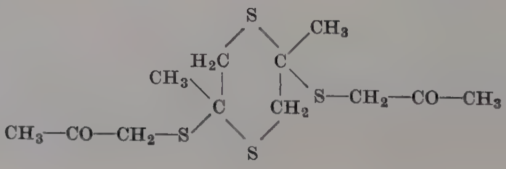
(XI)



(XII)



(XIII)



(XIV)

compound in question can readily be oxidized to mono- and disulfone with potassium permanganate in solutions of acetone and glacial acetic acid (5). HROMATKA and ENGEL have also tried to prepare the sulfones in a water suspension of the substance, the oxidizing agent being potassium permanganate and sulfuric acid. The yields are here considerably less, due, of course, to some hydrolysis, which was, however, not pointed out by the authors, who had not observed the relative ease of regaining the mercaptan. HROMATKA and ENGEL have carefully studied the behaviour of the sulfones, when being decomposed with alkali, and the results are in good agreement with the expected ones. Molecular weight determinations of the monosulfone, m.p. 104–105°, have been performed.

From the observations regarding the oxidation the presence of a disulfide link can be discounted and structure (XII) is thus eliminated. The absence of free mercapto groups can be proved simply, and the formulae (X and XI) must also be discounted. This means on the other hand that the statements of BAKER and BARKENBUS must be wrong on this point, a conclusion also arrived at by HROMAKTA and ENGEL. A further proof can be given since a derivative of the dithiene (XIII) has in fact been prepared by GROTH (1) in the following way. Two moles of water were removed from the dimeric mercaptoacetone, and an addition of the monomeric keto form of mercaptoacetone, present to some extent in the solution, to the double bonds was instantaneous, so the dithiene itself could not be isolated, and thus the derivative (XIV) was obtained. Among the remaining formulae, two, namely (VIII and IX) can be excluded from the results of HROMATKA and ENGEL on the basis of the behaviour of the sulfones, when they decompose with alkali. There are thus two remaining possibilities (VI and VII). The formula proposed by GROTH (VI) must give rise to a considerable strain in the molecule and it is hardly probable that an oxidation to sulfones can occur as no purely aliphatic ethylene sulfones are known (7). It has been established by SUNNER (8) on the other hand that the ethylene sulfide ring is markedly stabilized by replacing a hydrogen atom with a methyl group, but the oxygen bridge should not be overlooked as an important cause of instability. Further it seems rather improbable that a substance with such a structure would have a melting point above zero. The chemical properties of the compound are perhaps most consistent with formula (VII). It must be emphasized that even this structure cannot be quite strainless because of the oxygen bridge in the dithiane ring which might cause the observed hydrolysis. The fact that the substance can be prepared from the so-called BUNTE-salt — obtained from monochloroacetone and sodium thiosulfate — and from isomethylrhodime (5) is also in better agreement with the supposed dithiane structure. Determinations of the parachor and the molecular refraction support formula (VII), as the parachor is found to be 334.2 from measurements of the surface tension and the calculated values (9) are 349.2 for structure (VI) and 332.8 for structure (VII). The molecular refraction was calculated by HROMATKA and ENGEL from their formula to be 42.75, the observed value is 42.38, while 44.29 is obtained from GROTH's formula using the atomic refraction data of the literature (8, 10). Although it seems difficult to give conclusive evidence by purely chemical methods it is rather probable from the above consideration that the structure is that proposed by HROMATKA and ENGEL, thus 2.5-dimethyl-2.5-endoxy-1.4-dithiane (VII).

## Experimental

### Molecular weight determinations

**A. By the chryoscopic method:**

1. *Mercaptoacetone, dimeric,* m.p. 81–83°.
0.1168 g in 35.95 g bromoform: $\Delta t = 0.278°$
0.0889 g in 34.80 g » : $\Delta t = 0.217°$
M. W. calc. 180, found 169, 170.

2. *Mercaptoacetone, dimeric,* m.p. 107–108°.
0.0900 g in 35.56 g bromoform: $\Delta t = 0.215°$
0.2190 g in 33.07 g » : $\Delta t = 0.550°$
M. W. calc. 180, found 170, 174.

3. *Monosulfone of 2.5-dimethyl-2.5-endoxy-1.4-dithiane.*
0.1971 g in 35.25 g bromoform: $\Delta t = 0.409°$
0.1760 g in 31.85 g » : $\Delta t = 0.421°$
M.W. calc. 194, found 197, 189.

**B. By the ebullioscopic method:**

1. *p-nitrobenzoyl-mercaptoacetone.*
0.1271, 0.2637 g in 15.70 g benzene: $E = 0.082°$, 0.172°.
M. W. calc. 239, found 250, 247.

2. *Diacetonyl-disulfide dioxime.*
0.1128, 0.2132 g in 18.03 g acetone: $E = 0.050°$, 0.093°
M. W. calc. 208, found 214, 218.

### Acetonylmercapto-acetic acid

*A.* A solution of 13.9 g (0.10 mole) of monobromo-acetic acid in 30 ml of water was neutralized with 5.6 g (0.10 mole) of potassium hydroxide. 9.0 g (0.10 mole) of mercaptoacetone, (the amount calculated in regard to the monomer) dissolved in a solution of 5.6 g (0.10 mole) of potassium hydroxide in 40 ml of water, was added dropwise with cooling and stirring to the first solution and the mixture was stirred for another four hours. The solution was then acidified with concentrated hydrochloric acid and most of the water was removed *in vacuo*. The residue was extracted with ether, the ether layer separated and then dried with anhydrous sodium sulfate. The ether was evaporated and the residue distilled fractionally at reduced pressure. The yield of acetonylmercapto-acetic acid, boiling at 183–185°/11 mm, was 11 g. The acid crystallized upon cooling. The m.p. was 43–45° and the yield 75 %. This compound is very soluble in water, alcohol, ether and acetone, but almost insoluble in ligroin (b.p. 60–70°) and benzene.

*B.* 18.4 g (0.20 mole) of thioglycolic acid, dissolved in 25 ml of water, was neutralized with a solution of 22.4 g (0.40 mole) of potassium hydroxide in 30 ml of water. A solution of 18.6 g (0.20 mole) of monochloro-acetone in 20 ml of dilute alcohol (1: 1) was added drop by drop with cooling and stirring to the first solution and after four hours the reaction was complete. The mixture, which had been neutral, was made weakly alkaline and extracted with ether in order to remove any possible remaining monochloro-acetone. The ether layer was separated and the water layer was acidified by bubbling hydrogen chloride through the solution. The alcohol and most of the water was removed *in vacuo* and the acid was isolated in the same manner as in *A*. Its b.p. was 183–185°/11 mm, its m.p. 42–44° and the yield about 80 %.

*Anal.* Subst., 0.2554 g: 16.34 ml of 0.1049-N NaOH.
» 28.28 mg: $CO_2$, 41.62 mg; $H_2O$, 12.97 mg.
» 23.63 mg: $CO_2$, 34.87 mg; $H_2O$, 10.82 mg.
» 30.33 mg: 6.104 ml of 0.06725-N NaOH.
$C_5H_8O_3S$ (148.1) calc. Equ.wt. 148.1; C 40.57; H 5.44; S 21.64.
found Equ.wt. 149.0; C 40.14, 40.25; H 5.13, 5.12; S 21.70.

## X-ray photographs

Powder photographs were taken with $CuK_\alpha$-radiation, which was obtained using a monochromator of Guinier type (11). The time of exposure was two hours.

## Parachor and molecular refraction

The surface tension of the substance (VII), required for the parachor calculations, was determined by measuring the rise in a capillary tube, the diameter of which was found from experiments with liquids of known surface tensions, namely water, benzene, carbon tetrachloride and alcohol at the same temperatures. Values of refractive index and density are given by Hromatka and Engel (2).

The author is indebted to Professor Arne Fredga for valuable help and discussion.

## SUMMARY

Mercaptoacetone and its derivatives have been carefully studied and some discrepancies in the literature explained.

Acetonylmercapto-acetic acid has been prepared.

The structure of the compound $C_6H_{10}OS_2$ has been discussed and some arguments given to strengthen the formula proposed by Hromatka and Engel.

Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, February 1951.

REFERENCES. 1. **B. Groth**, Über α-Mercaptoketone und verwandte Stoffe. *Diss.* Uppsala (1926). — 2. **O. Hromatka** and **E. Engel**, Monatsh. *78*, 29 (1948). — 3. **H. Rheinboldt**, Ber. *60*, 184 (1927). — 4. **A. M. Drummond** and **D. T. Gibson**, J. Chem. Soc.

(London) *1926,* 3073. — 5. **O. Hromatka** and **E. Engel,** Monatsh. *78,* 38 (1948). — 6. **R. H. Baker** and **Ch. Barkenbus,** J. Am. Chem. Soc. *58,* 262 (1936). — 7. **B. Bäcklund,** Några α-haloalkylsulfoners reaktion med alkali. *Diss.* Uppsala (1945). — 8. **S. Sunner,** Studies in combustion calorimetry applied to organo-sulphur compounds. *Diss.* Lund (1949). — 9. **S. Sudgen,** The Parachor and Valency, p. 38, London (1930). — 10. Taschenbuch für Chemiker und Physiker. p. 1196, Berlin (1943). — 11. **A. Guinier,** Ann. phys. (II) *12,* 161 (1939).

Tryckt den 13 september 1951

Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 44

Vorgelegt am 14. März 1951

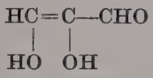
# Zur Kenntnis der Reduktone IX

Von Hans von Euler und Hans Hasselquist

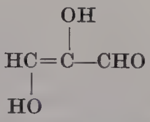
## 1. Reaktionsprodukte von (Triose-)Redukton A und (Triose-)Redukton II

Als Redukton A (Triose-Redukton A) bezeichnen wir im Folgenden das aus lukose in der von Euler und Martius[1] beschriebenen Form erhaltene Produkt $_3H_4O_3$ vom Schmpt 140–143°. Dieses Redukton A bildete zunächst das Ausangsmaterial für die Methylierung, die Acetylierung sowie für die Kondensation it aromatischen Aminen (Anilin, p-Nitranilin) aromatischen und aliphatischen minosäuren (p-Aminobenzoesäure, Sulfanilsäure: Leucin) ferner mit Hydrazinen, emicarbazid, Harnstoff u. a. Durch das Studium dieser Reaktionen des Reduktons haben wir eine Reihe von Erfahrungen, besonders reaktionskinetischer Art, emacht.

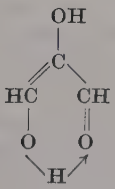
Geht man von der von Euler und Martius[1] vorgeschlagenen Strukturformel us, so ist eine cis-, trans-Isomerie des Reduktons zu erwarten:

cis-Form 

$$HC{=}C{-}CHO \quad (HO \text{ an } HC,\ OH \text{ an } C)$$

trans-Form 

Führt man, wie es F. Arndt und B. Eistert[2] vorziehen, eine Wasserstoffrücke ein, so ist letztere Formel etwa folgendermaassen zu schreiben:



Vor kurzer Zeit beschrieben Euler u. Hasselquist[3] eine Methode, aus dem edukton A mittels 1-Methoxy-butadien eine einheitliche Form abzuscheiden, arakterisiert durch den Schmpt 152–153°, welche nicht mehr mit dem Butadien agiert.

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[1] Euler u. C. Martius, Dieses Arkiv 11, No. 14 (1933). — Lieb. Ann 505, 73.

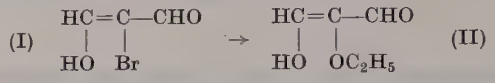
[2] B. Eistert, Dieses Arkiv 2, No. 6 (1950).

[3] Euler u. H. Hasselquist, Dieses Arkiv 2 No. 24 (1950). — Euler, H. Hasselquist u. Glaser, Dieses Arkiv 3, No 9 (1951).

Von den Redukton-Derivaten konnten bereits Fraktionen erhalten werden. welche als cis- und trans-Formen aufzufassen sind.

Was zunächst die Methyläther betrifft, so hat EISTERT bei ihrer Besprechung (l. c. S. 131) besonders den Einfluss von Methanol auf die Bildung des (nicht chelatisierten) Enol-methyl-äthers bezw. des Gemisches der beiden stereoisomeren Methyläther betont. In dem von uns isolierten Mono-methyl-äther dürfte die Methylierung in der 2-Stellung erfolgt sein.

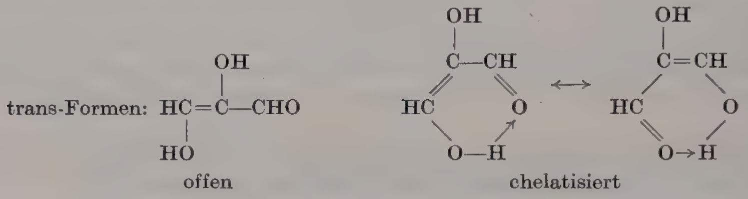
M. GRARD[1] hat aus der dem Redukton entsprechenden Br-Verbindung (I) den Äthyl-äther (II) gewonnen



$$\text{(I)}\quad \underset{HO}{HC}=\underset{Br}{C}-CHO \rightarrow \underset{HO}{HC}=\underset{OC_2H_5}{C}-CHO\quad \text{(II)}$$

Bei Verwendung von überschüssigem Diazomethan erhielten wir[2] ein öliges Produkt, welches nach EISTERT ein Gemisch der cis- und trans-Dimethyl-äther darstellt.

Behandelt man Redukton A (das Gemisch von cis- und trans-Redukton) mit 1-Methoxy-Butadien[2], so ist der mit dem Butadien *nicht reagierende* Anteil (Redukton II) die trans-Form:[2 3 4]



Dass trans-Redukton (in Abwesenheit starker Säuren) überwiegend in Ringform (chelatisiert) vorliegt, geht aus ihrem Verhalten gegen Ferrisalze hervor, wobe eine wesentlich schwächere Blaufärbung eintritt als mit Redukton A.

Wie W. COCKER[5] et al. betonen kann bei der cis-Form aus sterischen Gründe Chelatisierung nicht eintreten. Das Reaktionsprodukt der mit dem Dien reagierenden cis-Form des Reduktons haben wir bis jetzt noch nicht isolieren können. Wegen der principiellen Bedeutung des Reaktionsmechanismus solcher Endiol Philodiene setzen wir die Versuche zu ihrer Darstellung fort. Offenbar komm dem cis-Redukton eine hohe Reaktionsfähigkeit zu. Bei der Einwirkung vo Diazomethan auf *Redukton II* ergab sich: *Kurze* Einwirkung von überschüssige Diazomethan in ätherischer Lösung lieferte farblose Prismen (Sbst. HH 126) Schmpt 70–71°. — Bei *längerer* Einwirkung von überschüssigem Diazometha entstand ein Öl, dessen Methoxyl-Gehalt kleiner gefunden wurde als dem Dimethyl äther entspricht, ev. infolge seiner leichen Spaltbarkeit[6]. Der Mono-methyl-äthe des Reduktons II ist auch in fester Form nur wenige Tage haltbar.

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[1] M. GRARD, Ann. de Chim. (10. sér.) 13.336 (1930).

[2] EULER u. H. HASSELQUIST, Dieses Arkiv 3, No. 9 (1950–51).

[3] Die Bezeichnung cis und trans ist in der Litteratur der Reduktone bisher in verschiedne Weise zur Anwendung gekommen. Vgl. S. 1.

[4] Eine weitere Testreaktion für die cis-Form ergibt die Komplexbildung mit Borsäure.

[5] W. COCKER, R. A. Q. O'MEARA, J. C. P. SCHWARZ u. E. R. STUART, Chem. Soc. 1950, 2052

[6] Eine zur Wiederholung des Versuchs ev. geeignete Arbeitsweise bei der Methoxylbestimmun haben F. ARNDT u. F. NEUMANN angegeben. Ber. 70, 1937.

Einen wesentlichen Einfluss auf die Diazomethan-Reaktion scheint das Methanol zu besitzen, was mit den Erfahrungen von EISTERT an Acetylaceton (übereinstimmt.[1]

## Ester des Triose-Reduktons

Aus *Redukton A* wurde zunächst eine Mono-acetyl-Verbindung[2] $C_5H_6O_4$ vom Schmpt 101° erhalten. Ein entsprechendes Chloracetat wurde von EULER u. HASSELQUIST beschrieben; Schmpt 95–102°; nach 1 Min. an der oberen Schmelzgrenze: Schmpt 120–130°; es wurde charakterisiert durch die Reaktion mit o-Phenylendiamin, wobei das erwartete Chinoxalin-derivat entstand.

Ester des Reduktons mit 2-basischen Säuren: Siehe Dieses Arkiv 1, No. 63 und 2, No. 24 (1949).

Mono- und Di-benzoyl-Ester des Reduktons erwähnen COCKER, O'MEARA u. Mitarb. Chem. Soc. 1950, 2052. — Für Mono-benzoyl-redukton geben EULER u. HASSELQUIST den Schmpt. 148–149° an. 100 mg *Redukton II* (*trans-Redukton*) wurde nach dem gleichen Verfahren mit 0,4 ml Chloracetylchlorid umgesetzt. Die Ausbeute war nun 138 mg Ester vom Schmpt. 112–114°. Nachdem die Temp. 1 Min. bei 116° gehalten worden war, erstarrt die Schmelze, und ein neuer Schmpt: 146–148° tritt unter starker Zersetzung ein.

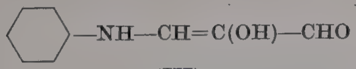
Bei Veresterung des Reduktons A mit 2 Mol Benzoylchlorid nach O'Meara erhielten wir beim Umkrystallisieren des Dibenzoylesters aus Alkohol abwechselnd höhere und tiefere Schmpte (z. B. gab eine Fraktion vom Schmpt. 121–122° (Analyse unten) beim Umkrystallisieren den Schmpt. 114–115°), vermutlich wegen Umlagerung Cis ⇄ Trans.

| | | | |
|---|---|---|---|
| *Analyse:* | Berechnet für $C_{17}H_{13}O_5$ | C = 68,91 % | H = 4,08 % |
| | Gefunden | 69,04 % | 4,42 % |

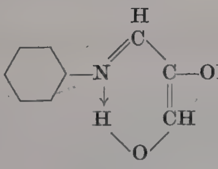
## Anilide (Anile) des Triose-Reduktons

EULER, HASSELQUIST und LÖÖV[3] erhielten durch Erhitzen von Redukton mit Anilin in stark salzsaurer Lösung ein HCl-Salz vom Schmpt. 260°. Sowohl aus diesem wie auch direkt (in Gegenwart von überschüssigem NaAc) konnte die freie Base in Form gelber Prismen vom Schmpt. 64° dargestellt werden.

COCKER ET AL. (l. c.) haben über die Redukton-Anilide mehrere von den unseren abweichende Angaben gemacht. Sie ziehen neben der offenen Formel (III) auch eine Chelat-Formel (IV) in Betracht



(III)



(IV)

Die Formulierung (III) stützen sie besonders auf ihre spektrophotometrischen Befunde.

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[1] B. EISTERT, F. ARNDT, L. LOEWE, u. E. AYCA, B. *84*, 156 [1951]; s. auch F. ARNDT u. B. EISTERT, Chem. Ber. 62, 36, (1929) — 68, 1572 (1935); F. ARNDT, L. LOEWE u. E. AYCA, Chem. Ber. 84, 333 (1951).

[2] EULER u. H. HASSELQUIST, Dieses Arkiv 2, No. 24 (1950).

[3] EULER, H. HASSELQUIST u. U. LÖÖV, Dieses Arkiv 26, No. 17 (1948).

Die genannten Forscher beschrieben auch das Hydrochlorid eines Dianils als gelbe Nadeln vom Schmpt. 245–255°, das sie durch Erhitzen von Redukton in saurer Lösung mit 1 oder 2 Mol. Anilin darstellten. Dasselbe dürfte dem von uns gewonnenen Di-p-nitranilid-methyläther entsprechen.

Eistert[1], der betont, dass alle bekannten Malonaldehyde mit Anilin glatt Mono-Anile geben, wendet auch für diese eine Chelat-Formulierung an, ,,die sie als vinylenhomologe Carbonamide mit in Scherenbindung befindlichem Proton erscheinen lässt''. ,,Mit dem Redukton — es handelt sich dabei nicht um das damals noch unbekannte Redukton II (trans-Redukton) — ,,liess sich bisher, entgegen anderlautenden Angaben englischer Forscher[2] auch mit überschüssigem Anilin-Hydrochlorid nur ein *Mono-Anil* bezw. dessen Hydrochlorid gewinnen''.

Wie Mono-methyl-anilin mit Redukton reagiert war hier noch nicht festgestellt worden. Redukton II (trans-Redukton liefert das gleiche Produkt wie von Cocker erhalten.

W. Cocker et al. (l. c. S. 2053) haben Methyl-anili als farblose Blättchen, Schmpt. 106–107° erhalten sie geben die Reaktion mit o-Dinitrobenzol[3] nicht.

Die in diesem Arkiv 3, No. 9 beschriebene Kondensation mit p-Nitranilin wurde nun mit Redukton II (*trans-Redukton*) wiederholt.

Aus 150 mg trans-Redukton und 236 mg p-Nitranilin wurden 317 mg Anilid erhalten; dasselbe stimmte sowohl bezgl. des Schmelzpunktes 232–233° wie auch bezgl. der Form und Farbe der Krystalle mit dem aus Redukton A gewonnenen Produkt überein.

## Kondensation des Reduktons A mit p-Bromanilin (HH 138)

2 g Redukton A in 100 ml $H_2O$ + 4 g p-Bromanilin in 200 ml verd. HCl-Lös. Gelbe Prismen. Beim Umkrystallisieren aus Alkohol ensteht ein unlösliches Pulver, Schmpt 274°. Verbraucht TR nicht. Das Hauptprodukt, dunkelgelbe Prismen, schmilzt bei 159,5°–160°.

| | | |
|---|---|---|
| *Analyse:* | Berechnet für $C_9H_8O_2NBr$ | Br = 33,01 % |
| | Gefunden | 33,14 % |

Es hat also 1 Mol Redukton mit 1 Mol p-Bromanilin reagiert.

## Kondensation des trans-Reduktons mit Semikarbazid

Diese früher (Arkiv 26 A No. 25; 1949) beschriebene Kondensation wurde mit 175 mg *trans-Redukton* und 250 mg Semikarbazid-chlorid unter Zusatz von HCl unter Stickstoff ausgeführt.

Die erhaltene Ausbeute, 64 mg betrug weniger als die Hälfte der mit Redukton A gewonnenen. Schmpt. 290°, Farbe und Löslichkeit des Produktes stimmen mit den früheren Beobachtungen überein. Es war also aus dem Isomeren-Gemisch offenbar das Semikarbazon der trans-Reduktons rein erhalten worden. Da diese Kondensation mit Redukton A in saurer Lösung schnell verläuft hatten wir in Betracht gezogen, dass in saurer Lösung die offene Aldehydform reagiert; für die in neutraler Lösung vorliegende Ringform dürfte die Chelatform die wahrscheinlichste sein[1]. Die Zwischenverbindung bei der Ringöffnung haben wir durch das Formelbild dargestellt:

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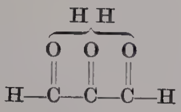
[1] B. Eistert, Dieses Arkiv 2, No. 6 (1950).

[2] H. S. Forrest a. J. Walker, Nature 161, 308 (1948). — Chem. Soc. 1949, 79 u. 2002.

[3] Wie F. Weygand (Dieses Arkiv 3, No. 2; 1950) erwähnt, ist diese Reaktion zuerst von R. Kuhn u. F. Weygand (Chem. Ber. 69, 1969; 1936) beschrieben worden.



$$\mathrm{H{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}H}\quad(\overbrace{\mathrm{O\;O\;O}}^{\mathrm{H\;H}})$$

## Kondensation von Redukton A mit Benzidin

### 1. 2 Mol Redukton und 1 Mol Benzidin. Sbst. HH 135

0.92 g feingepulvertes Benzidin wurden in 25 ml Wasser aufgeschlämmt und mit 3 ml 4-n HCl versetzt. Das Benzidin ging in der Wärme vollständig in Lösung. Zu der klar filtrierten Lösung wurde unter Umrühren eine filtrierte Lösung von 0.88 g Redukton in 10 ml Wasser zugegeben. Das Reaktionsprodukt krystallisierte sofort aus in Form intensiv roter, mikroskopischer Nadeln. Nach ½-stündigem Stehen wurden die Krystalle abfiltriert und succesive mit Wasser, Aceton und Äther gewaschen. Ausbeute 1.38 g (83 % der Theorie).

Bei 250° ging die Farbe der Krystalle in braun über. Der Schmpt. der Krystalle liegt jedoch nicht unter 295°.

Die Sbst. 135, welche halogenfrei befunden wurde, ist schwer löslich in den üblichen organischen Lösungsmitteln, unlöslich in Wasser, Alkali und in verdünnten Säuren. Sie löst sich in der Wärme in konz. Schwefelsäure mit brauner Farbe und fällt bei Zusatz von wenig Wasser wieder aus.

Suspendiert man die Sbst. 135 in 2-n NaOH und gibt Kaliumpersulfat zu, so bildet sich unter Oxydation eine ebenso schwer lösliche gelbe Verbindung.

### Titrierung der Sbst. HH 135 mit Dichlorphenol-indophenol (TR)

Die feinpulverisierte, in Wasser suspendierte Substanz verbraucht TR direkt, wenn auch wegen ihrer Schwerlöslichkeit langsam. Deswegen wurde die Titration in Stickstoff-Atmosphäre unter Zusatz von NaOH ausgeführt.

4,0 g feinst pulverisierte Sbst. in 10 ml Wasser wurden unter Stickstoff mit 2 ml 2-n NaOH versetzt und mit 0,01-n TR bis zur Entfärbung titriert. Verbrauch = 4,8 ml.

$$\begin{array}{ll} E_{\mathrm{ber}} \text{ für 1 Mol Benzidin + 2 Mol Redukton} = 324/4 & = 81 \\ E_{\mathrm{ber}} \text{ für 1 Mol Benzidin + 1 Mol Redukton} = 254/2 & = 127 \\ E_{\mathrm{gef}} & = 83 \end{array}$$

Mit 1 Mol Benzidin sind somit 2 Mole Redukton verbunden.

| *Analyse:* | | | |
|---|---|---|---|
| | Berechnet für $C_{18}H_{16}O_4N_2$ | C = 66,65 % | H = 4,97 % |
| | Gefunden | 66,74 % | 5,08 % |

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[1] Vgl. Euler u. Hasselquist: Reduktone (Verlag Enke, Stuttgart, 1949–50) S. 18 und 11.

### 2. 1 Mol Redukton und 1 Mol Benzidin. Sbst. HH 136

4,18 g Benzidin, in 100 ml Wasser suspendiert wurden nach Zusatz von 30 ml 2 n HCl erwärmt bis zur vollständigen Lösung, worauf warm filtriert wurde. Zu der noch warmen Lösung wurden 500 mg Redukton in 5 ml Wasser (filtriert) zugegeben. Nach etwa 1 Min. krystallisierte das Reaktionsprodukt in Form rotgelber Nadeln aus, die nach Abkühlung abfiltriert und mit Wasser und Aceton gewaschen wurden. Ausbeute 1 g. Bei 290° sintern die Krystalle unter Braunfärbung, schmelzen aber nicht. Die Substanz enthält Cl und liegt also als Chlorid vor, worauf auch die Löslichkeit in Wasser hindeutet.

Zur Darstellung der *freien Base* wurden 500 mg in 1000 ml siedenden Wassers gelöst, filtriert und die noch warme Lösung mit 10 ml einer gesättigten Natriumacetat-Lösung versetzt. Beim Erkalten fiel die freie Base in Form rotbrauner prismatischer Nadeln aus, die nach Abfiltrieren mit Wasser gewaschen wurden. Schmpt. 290°. Die Substanz war nun halogenfrei und schwerlöslich in allen üblichen organischen Lösungsmitteln, auch in Wasser sowie in verd. Säuren und Alkalien.

In 2-n NaOH aufgeschlämmt und mit Kaliumpersulfat versetzt, liefert die Sbst. 136 ein dunkelbraunes Oxydationsprodukt, zum Unterschied von Sbst. 135, aus welcher ein gelbes Produkt entsteht.

| | | | |
|---|---|---|---|
| *Analyse:* | Berechnet für $C_{15}H_{14}O_2N_2$ | C = 70,84 % | H = 5,55 % |
| | Gefunden | 69,43 % | 5,61 % |

### Titrierung der Sbst. HH 136 mit TR

3,95 mg Sbst. in 10 ml Wasser suspendiert wurden unter Stickstoff mit 2 ml 2-n NaOH versetzt und mit 0,01-n TR titriert. Verbrauch = 3,2 ml TR

$E_{\text{ber}}$ für $C_{15}H_{14}O_2N_2$ = 127 $E_{\text{gef}}$ = 123

### Kondensationsprodukte mit Aminosäuren

Aliphatische Aminosäuren wurden mit Redukton zuerst kondensiert von Euler, Hasselquist und Lööv[1], ferner von Cocker, O'Meara et al.[2] Auch hier dürften meist Gemische der stereoisomeren Formen vorgelegen haben.

Aromatische Aminosäuren des Reduktons wurden 1944 von O'Meara, McNally und Nelson[3] erwähnt. Sie wurden später — mit abweichenden Ergebnissen — von Forrest und J. Walker[4] kondensiert. Unsere mit Redukton A ausgeführten Versuche[5] stehen mit denjenigen von O'Meara et al. im Einklang.

Das aus Redukton A (erhalten aus dem Pb-Salz) mit p-Aminobenzoesäure dargestellte Produkt wurde von uns fraktioniert[6], wodurch ein gelbes und ein rotes Produkt $C_{10}H_9O_4N_2$ gewonnen wurde. Ihre Identifizierung mit den aus trans-Redukton dargestellten Kondensationsprodukten steht noch aus.

---

[1] Euler, H. Hasselquist u. U. Lööv, Dieses Arkiv 26 A No. 17 (1948).

[2] Angier et al. J. Am. Chem. Soc. 70, 25 (1948).

[3] E. A. Bell, W. Cocker, P. A. McNally a. H. G. Nelson, Biochem. J. 45, 373 (1949).

[4] R. A. Q. O'Meara, P. A. McNally a. H. G. Nelson, Nature, 154, 796 (1944).

[5] Euler u. H. Hasselquist, Rec. Trav. Chim. Pays-Bas 69, 402 (1950).

[6] Euler u. H. Hasselquist, Dieses Arkiv 3, No. 15 (1951).

## Zur Kinetik der cis- und trans-Reduktone

Das nunmehr leicht darstellbare trans-Redukton wird, besonders wenn es in seiner Chelatform vorliegt, dem cis-Redukton an Reaktionsfähigkeit unterlegen sein.

Wählt man zur Festlegung seiner Aktivität die für Reduktone charakteristische Eigenschaft, die Reduktionsfähigkeit, so sind die üblichen Testreagenzien. Dichlorphenol-indophenol, Jod und o-Dinitrobenzol wegen ihrer heftigen Einwirkung zu Messungen der relativen Reaktionsgeschwindigkeitem weniger geeignet als die Verfolgung der Aufnahme molekularen Sauerstoffs durch wässrige Reduktonlösungen mittels TR-Titration. Solche Versuche werden in diesem Institut mit verschiedenen aliphatischen und aromatischen (Adrenalin, Arterenol) Reduktonen ausgeführt.

Diese Reaktionen sind allerdings sehr empfindlich gegen Metall-Katalysatoren[1] besonders gegen $Cu^{++}$, weniger gegen $Fe^{+++}$. Silbernitrat wird in neutraler Lösung (pH 5,5–7,0) auch von trans-Redukton augenblicklich reduziert; wir untersuchen noch (unter Annahme der Chelatform), an welcher Stelle des Chelats die Salzbildung entsteht.

Das oxydo-reduktive Verhalten der beiden Redukton-Formen beruht also:

1) auf dem Grad der Komplex- bezw. Salzbildung einerseits der offenen Redukton-Formen und andererseits der Ringform (des trans-Chelates) mit katalysierenden Metallionen,
2) auf der Lage und der Beweglichkeit des Gleichgewichtes zwischen der offenen Kette und dem Ring (Chelat),
3) auf der Beweglichkeit des Gleichgewichtes

$$\text{cis-Redukton} \rightleftarrows \text{trans-Redukton}.$$

Frl. MARIANNE FREYNE danken wir bestens für eifrige Mitarbeit.

Stockholm, Universität, Vitamin-Institut u. Institut für organ.-chem. Forschung.

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[1] Zu dem S. 406 mitgeteilten Ergebnis bezgl. der Reaktion von trans-Redukton mit Ferrisalzen sei hier folgender Versuch angeführt: 5,9 mg trans-Redukton + 5 ml 96 %iger Alkohol + 1 ml 1 %ige alkoholische $FeCl_3$-Lösung. Schwache Blaufärbung; Entfärbungszeit: 1 Min. 31 Sek. 5,9 mg Redukton A. 5 ml 96 %iger Alkohol + 1 ml 1 %ige alkoholische $FeCl_3$ Lösung. Intensive Tiefblaufärbung. Entfärbungszeit: 69 Min. Siehe hierzu auch F. ARNDT, L. LOEWE u. E. AYCA, Chem. Ber. 84, 333 (1951).

Tryckt den 13 september 1951

Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 45

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# The loss of activity from blood cells and plasma which have been labelled with radioactive phosphorus ($P^{32}$)[1]

By GUSTAV NYLIN and GEOFFREY WADE[2]

With 8 figures in the text

## Introduction

The estimation of the circulating red cell volume by the use of blood activated with radioactive Phosphorus, a method first introduced by HAHN and HEVESY (2), depends on two fundamental assumptions; that complete mixing has occurred and that the number of labelled cells does not change significantly before sampling commences. NYLIN (10) has shown that the first assumption is correct but the validity of the second depends on two factors; possible loss of $P^{32}$ from the injected labelled cells, and if activated whole blood is injected, the penetration and labelling of further cells by $P^{32}$ contained in the plasma (4).

In this paper our purpose is to present some observations on the rate at which activity is lost from injected labelled cells, and upon the manner in which $P^{32}$ contained in injected plasma is lost from the circulation into the tissues, or taken up by circulating red cells. In this way we hope to obtain a clearer picture of the inherent accuracy of the technique, and be able to decide on the advisability of using either a suspension of activated cells in inactive plasma or saline (7, 14) or activated whole blood (11, 12, 13).

## Method

**1. General.** The method of activation and the preparation of samples for counting was that first devised by HEVESY and modified by NYLIN (11, 12). All samples were counted to a total of not less than 3.000 impulses.

Circulating red cell volume was calculated by the equation

$$V_R = Vo \cdot \frac{A\,1}{A\,2} \tag{1}$$

where $V_R$ = total circulating red cell volume, $Vo$ = volume of red cells injected,

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[1] We are indebted to Mrs GUNHILD LUNDBERG for financial help with the preparation of this paper.

[2] Dickonson Travelling Fellow in Medicine, University of Manchester.

$A1$ = the activity of the injected red cells, and $A2$ = the activity of the circulating red cells. (All activities are expressed in impulses/gram/minute).

Plasma volume was calculated by the equation

$$V_P = V_R \cdot \frac{(1 - Ht)}{Ht} \qquad (2)$$

where $V_P$ = plasma volume, $Ht$ = mean hematocrit. No correction was made for hematocrit error due to trapped plasma, or for the relationship between the venous hematocrit and the total body hematocrit.

The proportion of the $P^{32}$ contained in injected plasma remaining in the circulation at any given time ($a$) was calculated by means of the equation

$$a = \frac{A_P}{A0} \cdot \frac{V_P}{V_1} \qquad (3)$$

where $A_P$ and $A0$ are the activities of the circulating and injected plasma respectively, $V_P$ is the plasma volume and $V_1$ is the volume of activated plasma injected.

The proportion of injected plasma $P^{32}$ which might be expected to have penetrated the red cells at any given time ($b$) was derived from the equation

$$b = \left(a \cdot \frac{A_p}{A_2} \cdot \frac{Ht}{(1 - Ht)}\right) \qquad (4)$$

where $a$ is the proportion of the injected plasma $P^{32}$ still remaining in the circulating plasma, $A_P$ and $A_2$ are the activities of the circulating plasma and red cells and $Ht$ is the hematocrit. '$b$' is a theoretical estimation, for 'a' can only be derived when the blood volume is known whereas $A_2$ can only be obtained if activated plasma alone is injected, and this does not yield data for calculating the blood volume.

**2. Specific.** A. Ten experiments were performed on ten adults in whom the clinical examination and circulation time (decholin), venous pressure (9) and heart volume (8) were within normal limits. In each case blood was activated with 150 μC of $P^{32}$ in a negligible weight of $Na_2HPO_4$ and the cells were separated, washed once and then resuspended in inactive plasma, and injected. The activities of the circulating cells and plasma were followed for a period of seven days. Red cell and whole blood volume were calculated in each case by means of equations 1 and 2.

B. In four experiments on four normal cases, 10 ccs of plasma was separated by centrifugation, activated with 70 μC of $P^{32}$ and 4.5 ccs reinjected. In three of these experiments activities were followed in the circulating cells and plasma for 48 hours, and in the fourth for as long as they could be detected.

C. In four experiments, three normal cases and one in a case of congestive failure with cardiac enlargement and a raised venous pressure, 10 ccs of whole blood were activated with 100 μC of $P^{32}$ and 4.5 ccs were injected. Activities were followed in the circulating red cells and plasma by means of an indwelling needle in the brachial artery for a period of one hour. Red cell and plasma volumes were calculated and by means of equation 3 the rate of loss of $P^{32}$ from the plasma was obtained.

D. Four estimations of blood volume were made on each of two normal individuals. In each experiment the first two estimations were made by injecting activated cells suspended in inactive plasma while in the second two activated whole blood was used. All four estimations were made within a period of 2 hours.

## Results

Figure 1 is a mean curve constructed from the results of all 10 experiments and showing also the maximum scatter at each point of sampling. After 30 minutes the mean value was 94.8 % of the value at 5 minutes, the greatest fall being to 90.3 % and the least to 97.5 %. After 60 minutes the mean value was 91.6 % and the range between 87 % and 98 %. Within 48 hours 80 % of the activity had been lost and after 7 days it was barely detectable. Mathematically the curve can be divided into three parts, 0—1 hour, 1—48 hours, and 2—7 days, each part having an exponential form but with a different coefficient of rate of fall. Estimations on the circulating red cell volume gave figures ranging from 15.7 cc per kg body weight to 51.8 cc per kg body weight with a mean of 24.6. For total blood volume the figures range from 38.4 cc per kg to 126.3 with a mean of 61.9 cc per kg (Table 1).

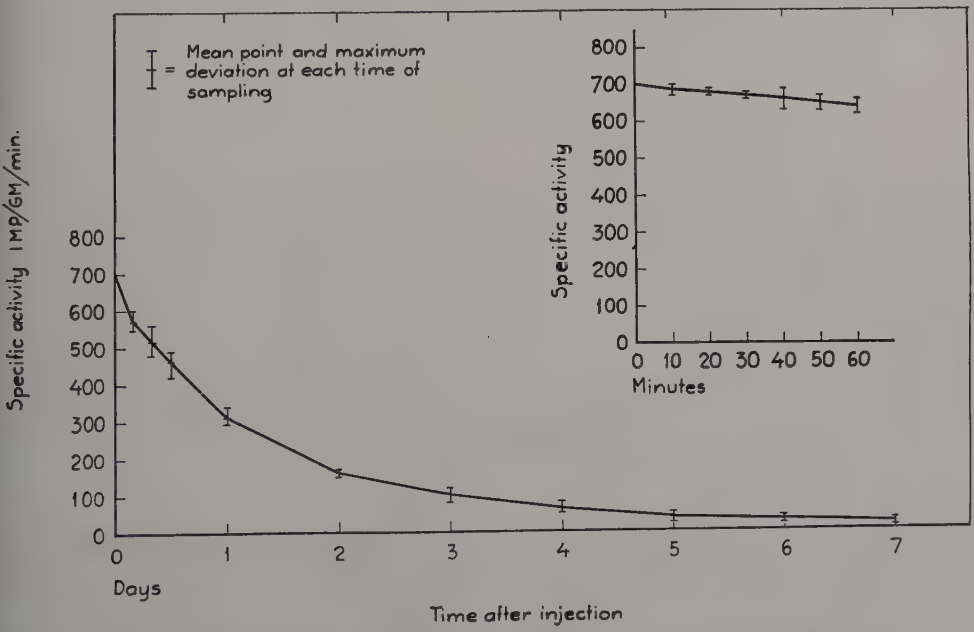


Fig. 1. The mean rate of fall of corpuscular activity, in 10 experiments, following the injection of activated red cells which have been washed once in inactive plasma. The scatter around the mean at each time of sampling is indicated by the vertical lines.

*Table I*

The circulating red cell, plasma and whole blood volumes, haemoglobin and red cell count in 10 cases.

| Case | Red blood corpuscles | | | | Plasma | | | | Circulat. blood volume | | | | Hb % | Red cell count (mill) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | gr | gr/kg | cc | cc/kg | gr | gr/kg | cc | cc/kg | gr | gr/kg | cc | cc/kg | | |
| 1 | 1260 | 20.2 | 1170 | 18.7 | 1870 | 30.0 | 1830 | 29.3 | 3130 | 50.2 | 3000 | 48.0 | 75 | 3.9 |
| 2 | 2470 | 30.0 | 2280 | 27.7 | 2420 | 29.4 | 2370 | 28.8 | 4890 | 59.4 | 4650 | 56.5 | 108 | 5.4 |
| 3 | 2100 | 30.9 | 1945 | 28.6 | 2685 | 39.5 | 2630 | 38.7 | 4785 | 70.4 | 4575 | 67.3 | 83 | 4.4 |
| 4 | 1310 | 20.6 | 1210 | 19.0 | 1570 | 24.6 | 1540 | 24.2 | 2880 | 45.2 | 2750 | 43.2 | 98 | 5.0 |
| 5 | 3610 | 56.0 | 3340 | 51.8 | 4900 | 76.0 | 4810 | 74.5 | 8510 | 132.0 | 8150 | 126.3 | 85 | 4.3 |
| 6 | 1540 | 19.0 | 1430 | 17.7 | 3250 | 40.2 | 3190 | 39.5 | 4790 | 59.2 | 4620 | 57.2 | 64 | 3.4 |
| 7 | 1160 | 18.1 | 1075 | 16.8 | 3470 | 54.2 | 3400 | 53.1 | 4630 | 72.3 | 4475 | 69.9 | 52 | 2.6 |
| 8 | 2360 | 31.3 | 2180 | 28.9 | 2410 | 31.9 | 2360 | 31.3 | 4770 | 63.2 | 4540 | 60.2 | 108 | 5.4 |
| 9 | 1470 | 17.0 | 1360 | 15.7 | 2000 | 23.1 | 1960 | 22.7 | 3470 | 40.1 | 3320 | 38.4 | 79 | 4.0 |
| 10 | 1045 | 23.1 | 970 | 21.4 | 1425 | 31.4 | 1395 | 30.8 | 2470 | 54.5 | 2365 | 52.2 | 94 | 4.7 |
| Mean | 1832.5 | 26.6 | 1696 | 24.6 | 2600 | 38 | 2548 | 37.3 | 4432 | 64.7 | 4245 | 61.9 | | |

Plasma activity never exceeded 5–6 impulses per gram per minute in any experiment and no deductions could be made from the form of the curve.

Figure 2 shows the results of all four experiments in which activated plasma only was injected. After 30 minutes plasma activity has fallen to approximately 50 % of its value at 5 minutes and after 60 minutes to 30 %. After 6 hours

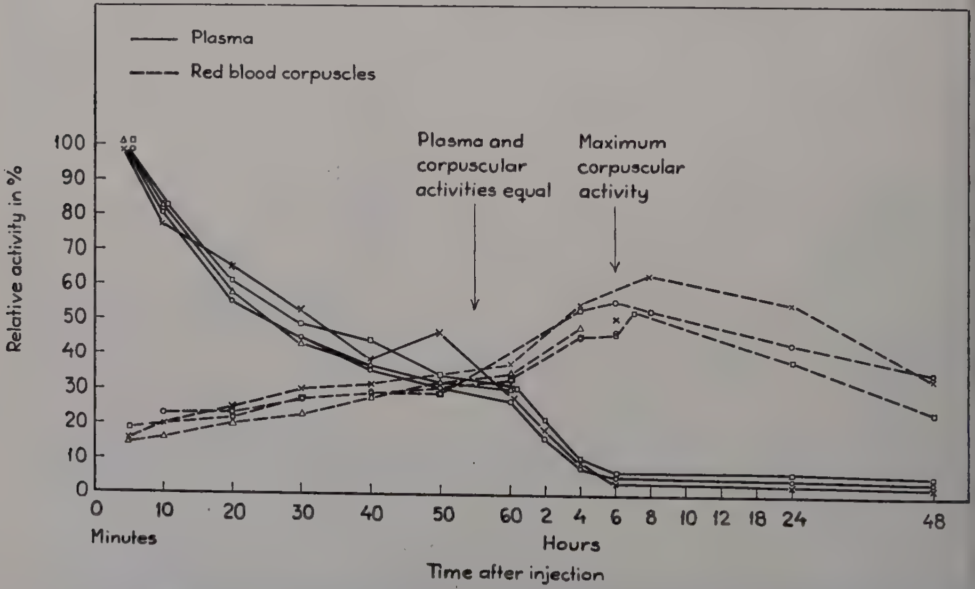


Fig. 2. The rate of fall of plasma activity and the increase in cellular activity from 5 minutes and onwards after the injection of activated plasma only (four expts).

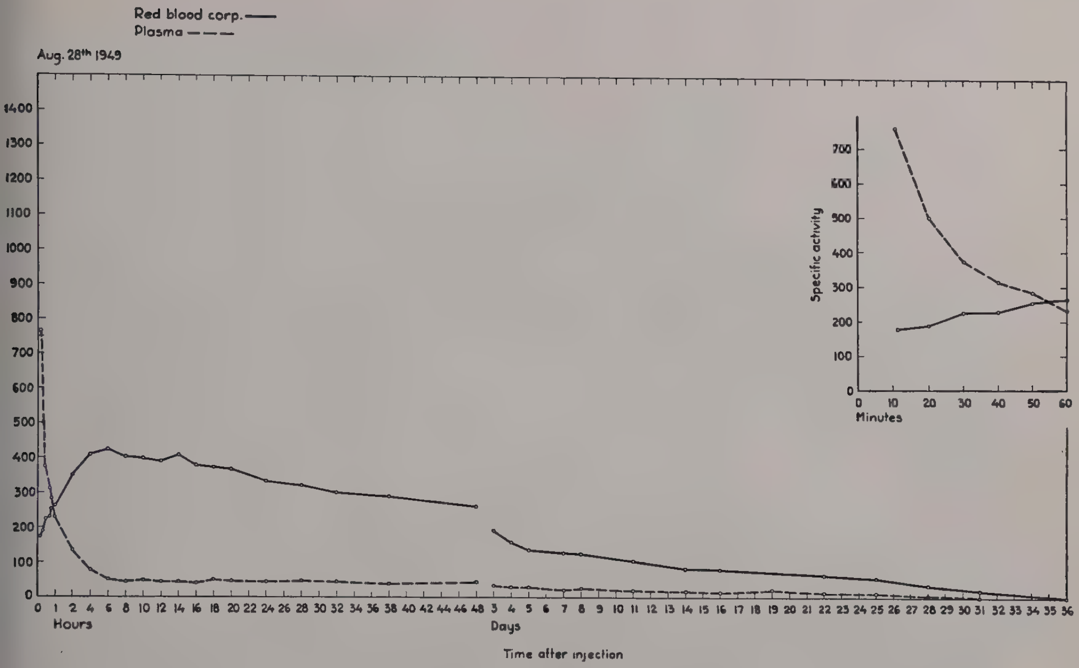


Fig. 3. Duration of persistence of activity of cells and plasma in a normal individual following the injection of activated plasma only.

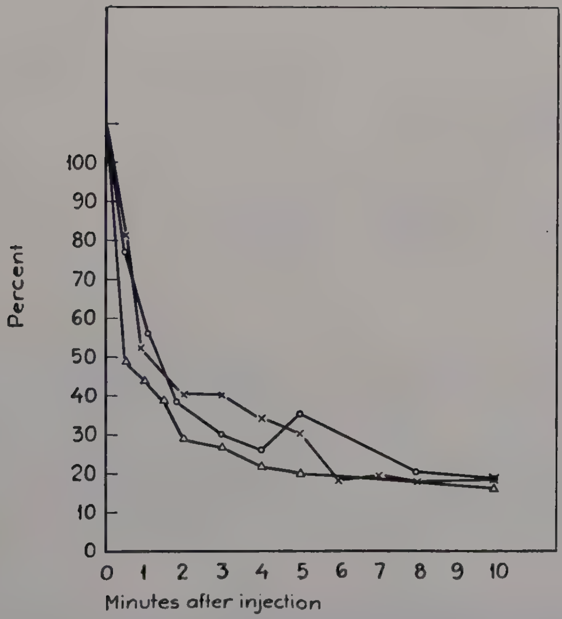


Fig. 4. The rate of loss of $P^{32}$ contained in injected activated plasma from the circulating plasma within the first 10 minutes in 3 normal individuals.

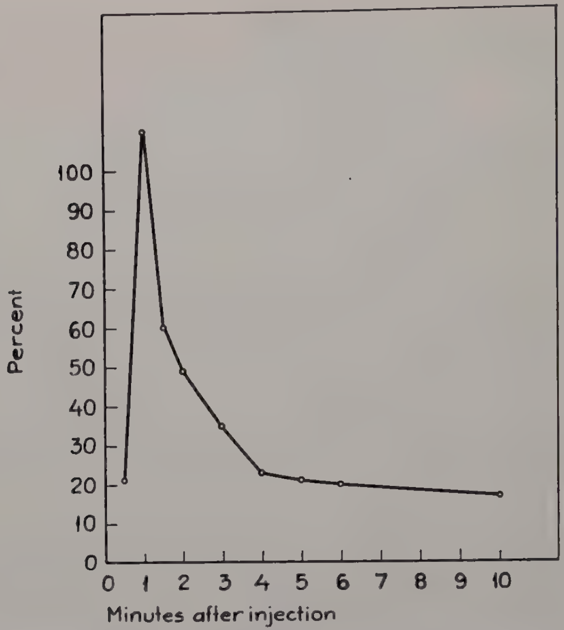
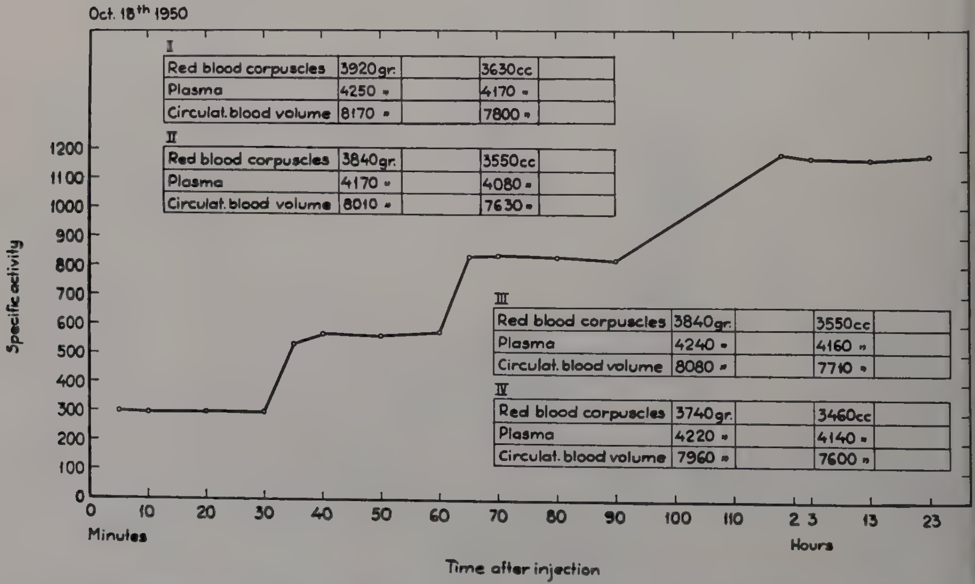


Fig. 5. The rate of loss of $P^{32}$ contained in injected activated plasma from the circulating plasma within the first 10 minutes in a case of congestive heart failure.



I

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 3920 gr. | | 3630 cc | |
| Plasma | 4250 » | | 4170 » | |
| Circulat. blood volume | 8170 » | | 7800 » | |

II

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 3840 gr. | | 3550 cc | |
| Plasma | 4170 » | | 4080 » | |
| Circulat. blood volume | 8010 » | | 7630 » | |

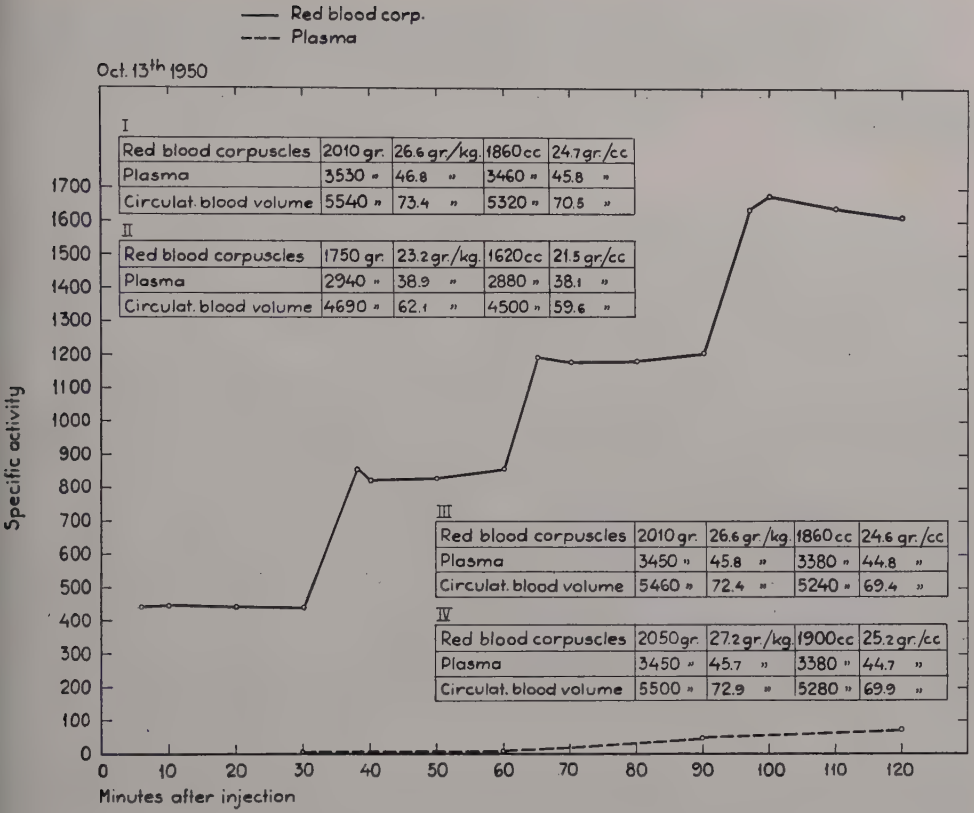
III

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 3840 gr. | | 3550 cc | |
| Plasma | 4240 » | | 4160 » | |
| Circulat. blood volume | 8080 » | | 7710 » | |

IV

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 3740 gr. | | 3460 cc | |
| Plasma | 4220 » | | 4140 » | |
| Circulat. blood volume | 7960 » | | 7600 » | |

Fig. 6. Consecutive estimations at half hourly intervals of the circulating red cell mass using washed cells for the first estimations and whole blood for the second pair.



I

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 2010 gr. | 26.6 gr./kg. | 1860 cc | 24.7 gr./cc |
| Plasma | 3530 » | 46.8 » | 3460 » | 45.8 » |
| Circulat. blood volume | 5540 » | 73.4 » | 5320 » | 70.5 » |

II

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 1750 gr. | 23.2 gr./kg. | 1620 cc | 21.5 gr./cc |
| Plasma | 2940 » | 38.9 » | 2880 » | 38.1 » |
| Circulat. blood volume | 4690 » | 62.1 » | 4500 » | 59.6 » |

III

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 2010 gr. | 26.6 gr./kg. | 1860 cc | 24.6 gr./cc |
| Plasma | 3450 » | 45.8 » | 3380 » | 44.8 » |
| Circulat. blood volume | 5460 » | 72.4 » | 5240 » | 69.4 » |

IV

| | | | | |
|---|---|---|---|---|
| Red blood corpuscles | 2050 gr. | 27.2 gr./kg. | 1900 cc | 25.2 gr./cc |
| Plasma | 3450 » | 45.7 » | 3380 » | 44.7 » |
| Circulat. blood volume | 5500 » | 72.9 » | 5280 » | 69.9 » |

Fig. 7 = 6.

the value was between 5–10 % and further loss was slight up to the 48th hour. Corpuscular activity increased rapidly during the first few minutes and then more slowly, equalling the plasma activity at the 55th minute and reaching a maximum at the end of 5 ½ hours when the ratio of activity in the cells and plasma was approximately 8–1. Thereafter corpuscular activity slowly fell, a new equilibrium being established after 48 hours with a cell plasma ratio of between 5–6 to 1. In one case we found that activity persisted in the cells for 36 days, in the plasma for 31 days and in the urine for 50 (Fig. 3).

The $P^{32}$ contained in the injected plasma disappears from the circulation very rapidly (Fig. 4). 50 % is lost within a minute and nearly 75 % within 5 minutes. In heart failure the loss is slower and the curve displaced to the right, although after 4 minutes an even higher proportion than usual has been lost and the slope of the curve between the 2nd and 4th minutes is consequently much steeper (Fig. 5).

Comparative estimations of red cell volume using washed cells and whole blood agreed in the first case (Fig. 6) to within 5 %. The mean of the two estimations obtained with washed cells was 3 % greater than the mean of the two readings obtained with whole blood (Fig. 6). In the second experiment (Fig. 7) the first, third and fourth estimations corresponded to within 1.5 % but the second estimation was approximately 12 % lower. The mean of the estimates obtained with whole blood was 6.5 % greater than those obtained with washed cells.

## Discussion

The fall in activity of the labelled red cells of approximately 5 % within 30 minutes and 8 % within 60 minutes accords with the findings of HEVESY and ZERAHN (4) and KELLY, SIMONSON, and ELMAN (7), and will cause a corresponding overestimate of the circulating red cell volume. This error will be the less the sooner sampling is completed.

HEVESY and ATEN (3) showed that the rate of corpuscular activation is proportional to the specific activity of the cells and plasma but that ingress will be more rapid than egress owing to the much greater amount of soluble phosphate in the cells than in the plasma (approximately 10: 1). BOHR (1) has calculated the penetration constant for $P^{32}$ and found it to be 0.37; the constant for loss of $P^{32}$ from the cells he found to be 0.03, figures which correlate well with the relative amount of soluble phosphate in the cells and plasma. Thus penetration of the red cells by $P^{32}$ contained in the injected plasma will occur mostly during the first few minutes, although equilibrium will not be achieved until the plasma activity has fallen to approximately 1/10 of that of the red cells. A theoretical curve constructed from equation 4 agrees with this, between 3–3.5 % of the total activity contained in the injected plasma penetrating the cells within 10 minutes, 5 % within one hour and a maximum of 8.5 % at 6 hours (Fig. 8), figures which agree with those of HEVESY, KÖSTER, SÖRENSON, WARBURG and ZERAHN (6). After the 10th minute the rate of increase does not therefore equal the rate of loss of activity from the red cells, when activated whole blood is injected.

The magnitude of this effect on volume determinations when activated whole blood is used, depends on the relative proportions of $P^{32}$ contained in the cells and in the plasma. Assuming a partition coefficient of unity and hematocrit of 50 the amounts will be equal; with a coefficient of 0.75 and an hematocrit of 30 there will be over 3 times as much $P^{32}$ contained in the plasma as in the cells and the magnitude of the penetration in relation to the activity of the cells would be correspondingly higher. Activating for 2 hours at 37° C we have found coefficients ranging between 0.7 and 1.4 and the hematocrit has a wide variation both in health and disease. Time may also play a role; we have showed that, owing to slow mixing in heart failure, high plasma activities persist for a longer time, although the later rate of fall may be more rapid, and to a lower level, because of the larger extracellular fluid volume. When mixing is slow, therefore, the degree of penetration may be variable, although the direction of the change cannot be predicted.

These considerations would lead us to expect slightly lower figures for red cell volume when activated whole blood is injected by contrast with the figures obtained when washed cells are used. Fig. 6 shows that in one of the two cases in which we made four volume estimations this obtained. In the second (Fig. 7) the figures for whole blood are 6.5 % greater than the mean of the

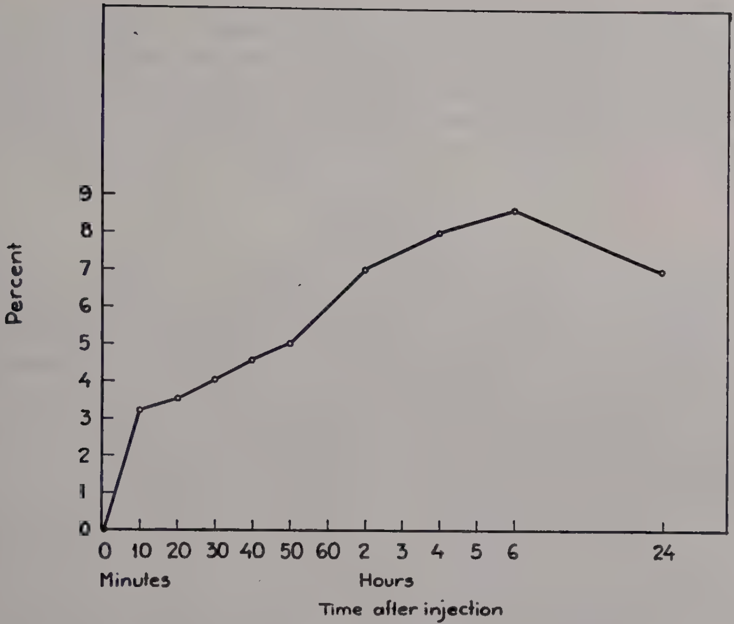


Fig. 8. The proportion of $P^{32}$ contained in the plasma portion of injected activated whole blood which may be expected to penetrate hitherto unmarked circulating red cells. (For explanation of the method of derivation, see text).

results with washed cells due to an exceptionally low reading at the second estimation. Examination of the samples taken between the 30th — 60th minutes shows a rising activity instead of the expected 5–6 % fall and as the plasma activity was nil during this period, this must be due to experimental error. In both these experiments the partition coefficient of the activated blood was greater than 1 while the hematocrit was also high and thus the proportion of radioactive phosphorus contained in the injected plasma was not excessive in either case.

The red cell mass in the 10 cases of the first experiment, 26.6 grams/kg body weight, is lower than the figure of 31.2 grams/kg body weight, found by Nylin and Hedlund (12) in 19 normal individuals, but reference to table I shows that this may be partly due to a mild anaemia present in 3 cases.

The gradual increase of cellular activity following the injection of activated plasma, reaching a maximum at between 5–6 hours with a cell/plasma ratio of approximately 8: 1 agrees well with Bohr's (1) findings. The ensuing fall, with the establishment after 48 hours of a new equilibrium in the proportion of between 5–6: 1, suggests that some of the $P^{32}$ in the plasma is now in a form which cannot penetrate the cells. Hevesy and Aten (5) have shown that hexosemonophosphate does not penetrate the cell membrane and it is possible that some of the $P^{32}$ is now present in this form, or that it has been utilized in the liver in the building up of phospholipids.

The three factors concerned in the loss of activity from activated cells cannot be ascertained with certainty. The deeper penetration of the phosphorus contained in the cell, with the formation of non-hydrolizable acid soluble phosphorus compounds (3) may be largely responsible. The striking prolongation of cellular activity when active plasma is injected is probably due to continued penetration of new cells by $P^{32}$ from the plasma and possibly, also the utilization in the erythropoietic process of phosphorus compounds containing $P^{32}$.

## Conclusions

We may therefore conclude that although radioactive phosphorus contained in injected plasma is rapidly lost from the circulation a significant degree of penetration of the red cells may occur. Assuming a partition coefficient between the cells and the plasma of the injected blood of unity, and the hematocrit within the normal range this will not exceed the activity lost from the cells (about 3–5 % within 30 minutes), but with a low partition coefficient and hematocrit value it may be considerably increased. Because of the variability both of coefficient and hematocrit we think that the use of activated washed cells is preferable to whole blood in the estimation of blood volume. To avoid significant error due to loss of activity from the cells we suggest that sampling should be completed within 30 minutes of injection and under these circumstances the inherent error of the method is within 5 %.

The decreased dose of radioactivity injected and the shorter time of persistence are also arguments in favour of using washed cells, and the technique will permit of a shorter activation time.

The uptake of $P^{32}$ from the plasma by the cells follows BOHR's (1) predictions, but the change in equilibrium occurring between 6 and 48 hours with a consequent change in the penetration coefficient suggests that some of the $P^{32}$ in the plasma is being incorporated in compounds which do not penetrate the cell membrane.

## SUMMARY

1. The rate at which activity is lost from injected red blood cells labelled with radioactive phosphorus and the way in which radioactive phosphorus contained in injected plasma is lost from the circulation has been studied in normal individuals.

2. Cellular activity was found to fall by an average of 5.3 % within 30 minutes, 8 % within 60 minutes, 55 % within 24 hours and to have almost disappeared within 7 days. There are three factors in the curve of loss presumably related to the deeper penetration of the contained $P^{32}$.

3. 50 % of the total amount of radioactive phosphorus contained in the injected plasma was removed from the circulation within one minute, 75 % within 5 minutes, and 95 % within one hour. Slight activity persists over 30 days. Uptake by the red cells is slow reaching a maximum at 6 hours when approximately 8 % of the total radioactive phosphorus contained in the injected plasma has entered the cells. After one hour 5 % has entered, an amount corresponding closely to that lost from the cells.

4. The effect of the penetration of cells by $P^{32}$ from the plasma on the estimation of blood volume is discussed. It is concluded that under certain

circumstances the use of washed cells is preferable and that provided sampling is completed within 30 minutes of injection the method will measure the circulating red cell volume within an accuracy of within 5 %.

5. In 10 individuals, using activated washed cells we found the mean red cell volume to be 24.6 cc per kg body weight and the mean whole blood volume to be 61.9 per kg body weight. A mild anaemia in 3 cases was considered to be partly responsible for the low figures.

## Acknowledgements

We wish to thank professor G. HEVESY for his unfailing help and advice. We also wish to thank Mrs A.-L. GUSTAFSSON and Miss M. LINDBERG for their technical help with the preparation and counting of the samples.

REFERENCES. 1. **Bohr, H. H.,** Kgl. Danske Vidensk. Selskab. Biol. Med. 1950: XVIII: 1: 1. — 2. **Hahn, L.,** and **Hevesy, G.,** Acta Physiol. Scand. 1940: 1: 3. — 3. ——, Acta Physiol. Scand. 1941/42: 3: 193. — 4. **Hevesy, G.,** and **Zerahn, K.,** Acta Physiol. Scand. 1942: 4: 376. — 5. **Hevesy, G.,** and **Aten, A.,** Kgl. Danske Vidensk. Selskab. Biol. Med. 1939: 14: 5. — 6. **Hevesy, G., Köster, K. H., Sörenson, G., Warburg, E.,** and **Zerahn, K.,** Acta Med. Scand. 1944: 116: 561. — 7. **Kelly, F. J., Simonsen, D. H.,** and **Elman, R.,** J. Clin. Invest. 1948: XXVII: 795. — 8. **Liljestrand, G., Lysholm, E., Nylin, G.,** and **Zachrisson, C. G.,** Amer. Heart J. 1939: 17: 406. — 9. **Moritz, F.,** and **Tabora, D.,** Deutsches Arch. f. Klin. Med. 1910: 98: 475. — 10. **Nylin, G.,** Amer. Heart J. 1945: 30: 1. — 11. ——, Ark. Kemi, Min., Geol. 1945: 20: 17: 11. — 12, **Nylin, G.,** and **Hedlund, S.,** Amer. Heart J. 1947: 33: 770. — 13. ——, Amer. Heart J. 1949: 37: 543. — 14. **Reeve, E. B.,** and **Veall, N.,** J. Physiol. 1949: 108: 12.

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Read 25 April 1951

# Thorium B labelled red corpuscles

By G. HEVESY

The labelling of red corpuscles is an important application of radioactive indicators as labelled erythrocytes are applied among others in circulation studies and in the determination of blood corpuscle (blood) volume.[1]

Labelling of red corpuscles with $^{32}P$ is made possible by the presence of comparatively large amounts of labile acid soluble phosphorus in the erythrocytes. Much of the $^{32}P$ which intrudes from the plasma into the red corpuscles speedily participates in the glycolytic and other enzymatic processes taking place in the erythrocytes. This participation involves incorporation into labile organic phosphorus compounds and thus "dilution" of the intruded $^{32}P$. As a result of this "dilution" if in the course of 1 hour 1 mg intruding phosphorus carries 1 μcurie into the corpuscles 1 mg phosphorus moving from such corpuscles in the opposite direction carries into inactive plasma an appreciable smaller activity which amounts to about $^1/_{12}$ of 1 μcurie only. Interchange of phosphate between plasma and red corpuscles is a continuous process not influenced by the presence of added labelled phosphate, the latter having a negligible weight. Injected into the circulation such labelled red corpuscles will lose 8 per cent of their activity in the course of 1 hour. In the course of 20 minutes the loss is certainly less than 3 per cent. This time interval is in most cases sufficient to carry out circulation velocity or blood volume measurements.

In a similar way red corpuscles can be labelled by adding $^{42}K$ to a blood sample and shaking it in a thermostat for an hour or two.[2] In this case — due to the much higher potassium content of the erythrocytes — the intruded $^{42}K$ becomes "diluted" by endogenous potassium in a similar way as intruded $^{32}P$ gets "diluted" by endogenous phosphorus and correspondingly the $^{42}K$ labelled corpuscles injected into the circulation lose less than 4 per cent of their activity in the course of 1 hour.

In some pathological cases with impaired circulation mixing of the injected and circulating red corpuscles takes place at a much slower rate than under physiological condition and accordingly it may be desirable to follow the path of the labelled erythrocytes in the circulation for many hours.

Such observations can not be carried out by making use of $^{32}P$ or $^{42}K$ labelled red corpuscles.

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[1] cf. G. NYLIN, Svenska Vetenskapsakad. Arkiv Kemi, A 20, No. 17 (1945).

[2] G. HEVESY and G. NYLIN, Acta Physiol. Scand., in print.

Recently $^{51}$Cr was suggested as labelling agent of erythrocytes. Washed red corpuscles, labelled with $Na_2{}^{51}CrO_4$ in vitro, were injected intravenously into dogs and were found to retain their activity without significant loss to the plasma for approximately 24 hours. The previously determined red corpuscle volume could be re-determinated within 5 % for approximately 24 hr.[1]

Neither β-particles nor positrons are emitted by $^{51}$Cr but x-rays produced through internal conversion. These can be measured with great efficiency by making use of a crystal counter which, however, requires expert assistance, in contrast to the running of a Geiger counter. Future progress in the measuring technique may eliminate the difficulties encountered today by a clinical institution when trying to apply red corpuscles labelled with $^{51}$Cr in corpuscle volume measurements.

In this note a labelling method of red corpuscles is described which permits to produce tagged erythrocytes which in the course of many hours lose only minute amounts of their radioactivity.

The method is based upon the introduction of thorium emanation (thoron) into the blood sample. The emanation, which has a half-time of 55 sec, penetrates speedily into the red corpuscles, decays inside them producing the active deposit of thorium which remains even for many hours to a very large extent fixed in the red corpuscles.

The sequence of the radioactive disintegration products of thorium emanation (Tn) is the following:

$$\begin{array}{ccccccccc} \text{Tn} & \longrightarrow & \text{ThA} & \longrightarrow & \text{ThB} & \longrightarrow & \text{ThC} & \longrightarrow & \text{ThC}' \\ 55\,\text{sec} & & 0.16\,\text{sec} & & 10.6\,\text{hr.} & & 60.5\,\text{min} & & \\ & & & & & & \downarrow & & \\ & & & & & & \text{ThC}'' & & \\ & & & & & & 3.1\,\text{min} & & \end{array}$$

The ThA decays with a half-time 0.16 sec and can thus be disregarded. ThB, however, which has a half-time of 10.6 hr., comes soon in exchange equilibrium with the following disintegration products. Consequently the activity of the erythrocytes decays with the disintegration period of ThB. This radioactive body emits soft β-rays, its disintegration products hard β-rays. One of these ThC″ emits furthermore very hard γ-rays. The approximate half-value thickness of the β-rays emitted by ThB and its disintegration products in aluminium is the following:

ThB (lead isotope) $1.6 \times 10^{-1}$ mm
ThC (wismuth isotope) 1.2 mm
ThC″ (thallium isotope) $7.7 \times 10^{-1}$ mm

The half value thickness of the γ-ray emitted by ThC″ is 17 cm.

As the β-rays emitted by ThC have almost the same hardness as those emitted by $^{32}$P, they are very conveniently measurable.

In our experiment a stream of oxygen after having brushed over the surface of a radiothorium preparation of about 1 mC activity was lead through 4 ml of rabbits blood. Plasma and red corpuscles were then separated, washed 2 to 3 times with inactive plasma and then shaken with inactive plasma of the same rabbit for 2 hr. at 37° in the thermostat.

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[1] S. J. Gray and K. Sterling, 1950 (A. E. C. U. — 1027). — K. Sterling and S. J. Gray, 1950 (A. E. C. U. — 1026).

Corpuscle and plasma samples were dried at 60° and 50 mg of each sample placed in an aluminium dish; their β-activity was then determined with the Geiger counter. We determined the water content of each corpuscle and plasma sample and could thus calculate from the activity figures of the dry samples the activity of fresh plasma and fresh corpuscles.

The thorium emanation penetrates rapidly into the red corpuscles. It decays very rapidly as well and we soon find 1 g of corpuscles to contain twice or more of thorium active deposit than 1 g of plasma. These figures depend on the velocity of the oxygen stream and on other experimental conditions. Through decay of thoron thorium B is formed in the plasma as well and a part of this thorium B i also taken up by the erythrocytes.

We found that after 2 hr. shaking of active corpuscles with inactive plasma the corpuscles contained 50 560 counts but the plasma contained only 170. Thus the red corpuscles gave off only 0.34 % of their activity in the course of 2 hr. The maximum loss was shown by a corpuscle sample containing 87 800 counts, with 403 counts in the corresponding plasma. In this case the loss was 0.46 %.

All these activities were measured 24 hr. after leading thoron into the blood, thus after more than 2 decay periods of ThB have already elapsed. Strong corpuscle activities can thus be obtained by leading thoron for a few minutes only through a blood sample.

That the erythrocytes so well retain their content of ThB and its disintegration products is partly due to the fact that much of the active deposit of thorium present in the red corpuscles is in a colloidal or protein bound state. Much of the decay of thorium emanation leads to formation of such thorium B particles.

That much of the activity of the red corpuscles was due to colloidal or protein bound particles was shown by making use of the fact[1] that on a zinc plate dipped into a solution containing colloidal ThB (lead isotope) or ThC (wismuth isotope) much more activity accumulates when acidifying the solution thus converting the radiocolloids into ions.

When 0.5 g of corpuscles were hemolyzed by adding the same volume of water and one half of that volume was shaken after immersion of a zinc plate (2×2 mm) for 5 minutes, the washed zinc plate had an activity of 3540 counts per min. When first adding 0.5 ml of 1 n HCl to the other half of the hemolysate and then repeating the experiment, the zinc plate had an activity about 4 times as large namely 14 897 counts per min than in the first mentioned experiment. The addition of acid converted much of the colloidal and protein bound active deposit into the ionic state. It follows that about 3/4 of the active deposit present in the corpuscles was in a colloidal or protein bound state.

The results of blood volume determinations obtained by NYLIN and the present writer in an investigation in which thorium B labelled red corpuscles were applied will be shortly published.

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[1] G. HEVESY, Sitzungsber. Wiener Akademie der Wiss. 127, Abt 2 a. (1918).

### SUMMARY

By leading thoron (thorium emanation) through blood for a few minutes red corpuscles labelled by the presence of the active deposit of thorium are obtained.

The red corpuscles of the rabbit conserve their activity for many hours, less than 1/2 per cent being lost when shaking the active erythrocytes with inactive plasma for 2 hr. at 37°.

The authors best thanks are due to Miss GRACÉ DÉ ELLIOTT for her very effective assistance.

Institute for Research in Organic Chemistry, University of Stockholm.

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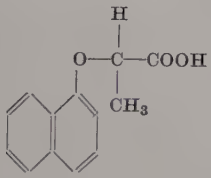
# Studies on synthetic growth substances. II.[1] The steric relations between the optically active naphthoxy-propionic acids

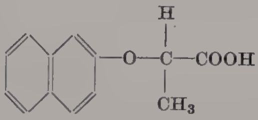
By Arne Fredga and Magnus Matell

With 4 figures in the text

Recent investigations by Dr. Börje Åberg have shown, that the optical antipodes of α-(1-naphthoxy)-propionic acid (I) have different growth-regulating activity, the (−)-form being more effective. The α-(naphthoxy)-propionic acid (II) is still more effective and the difference between the enanthiomorphs is far more pronounced. Here, however, the (+)-form is more active, acting as a typical auxin, while the (—)-form is an auxin antagonist. These experiments are described by Dr. Åberg in a separate publication (1).



(I)



(II)

Recently, Wain has reported experiments with α-(2-naphthoxy)-propionic acid (II), using other tests, and found a striking difference in the activity of the enanthiomorphs (2). Wain also draws attention to the fact, that the naphthoxy-acetic and the naphthoxy-propionic acids are highly active, while the naphthoxy-*iso*-butyric acid is inactive. It is interesting to note, that one of the antipodes of the propionic acid derivative is analogous to the acetic acid, while the other enanthiomorph behaves like the *iso*-butyric acid. Thus, in this case the presence of one methyl group in a certain sterical position seems to be sufficient to destroy the auxin activity.

For a better understanding of the influence of sterical factors it is essential to know if the (—)-form of (I) and the (+)-form of (II), which are more effective than their respective antipodes, have identical or opposite configurations of the side chain. It is well known that no definite conclusions can be drawn from the directions of rotation, and it is often very difficult to establish the steric relationships.

In 1895, Winther advanced the theory, that the enanthiomorphs of different

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[1] First communication: Arkiv Kemi *3* (nr 16), 123 (1951).

*Table 1*

$[\alpha]_D^{20}$ for (+)-1-naphthoxy-propionic acid (I) and (—)-2-naphthoxy-propionic acid (II) in different solvents

| | I | II |
|---|---|---|
| Acetone | +47.2° | —108.8° |
| Alcohol (abs.) | +49.6° | —93.3° |
| Chloroform | +58.8° | —62.1° |
| Acetic acid | +73.1° | —119.1° |
| Water ($NH_4$-salt) | +95.9° | —73.4° |
| Benzene | +149.6° | —94.3° |

acids, which are precipitated by the same alkaloid, are sterically related ("Winther's rule") (3). In this very general formulation, the rule is certainly not valid, but if the acids have similar structures, it may be of limited application. The racemic naphthoxy-propionic acids have been resolved by FOURNEAU and BALACEANO, using cinchonine (4). As a matter of fact, the (+)-form of (I) and the (—)-form of (II) are isolated using their sparingly soluble cinchonine salts, while their antipodes can be obtained from the mother liquors. This would indicate, that the acids having different directions of rotation have the same configuration.

It is often possible to establish steric relations with a high degree of probability by comparing the rotatory powers of the two substances through a series of derivatives or in a number of different solvents. In table 1, the activity data for different solvents are tabulated to give an increasing series for (+)-α-(1-naphthoxy)-propionic acid. The values for the 2-naphthoxy derivative, however, will then vary quite irregularly. It is obvious that this method gives no information in the present case.

The phase diagrams for systems of optically active compounds will often give useful information as to configurational relations. Sterically related compounds give simple eutectic diagrams or solid solutions, while compounds of opposite configurations may form quasi-racemic compounds (5). As seen from fig. 1, the inactive α-(1-naphthoxy)-propionic acid is a true racemic compound, melting higher than the antipodes. The inactive 2-naphthoxy acid, which has a lower melting point than the enanthiomorphs, is also a true racemic compound; a closer investigation revealed the existence of three polymorphic modifications. The melting point diagrams of two of them could be studied (fig. 2), but the third modification is too unstable. 1- and 2-naphthoxy-propionic acids having the same direction of rotation give a molecular compound 1:1, while the acids of different directions of rotation show a diagram of the common eutectic typ (fig. 3 and 4). The existence of the molecular compound could also be established by studying the system using KOFLER's contact method (6). The formation of this compound, which is most probably a quasi-recemic compound, is a very strong argument in favour of the opinion, that the acids with different directions of rotation have the same configuration. A study of the arrangement of the acid molecules in the crystal lattices of the quasi-racemic compound and the two true racemic compounds would in this case be of special interest.

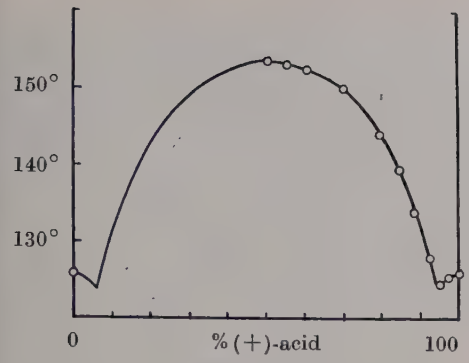


Fig. 1. (+)- and (—)-α-(1-Naphthoxy)-propionic acid.

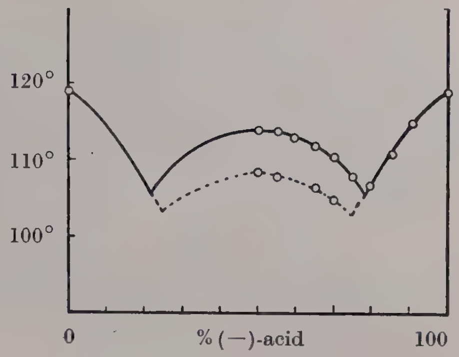


Fig. 2. (+)- and (—)-α-(2-Naphthoxy)-propionic acid.

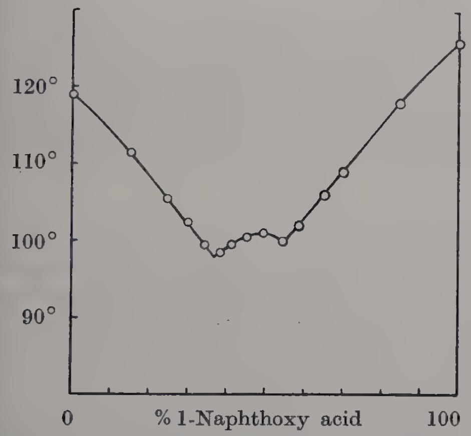


Fig. 3. (—)-α-(1-Naphthoxy)-propionic acid and (—)-α-(2-Naphthoxy)-propionic acid.

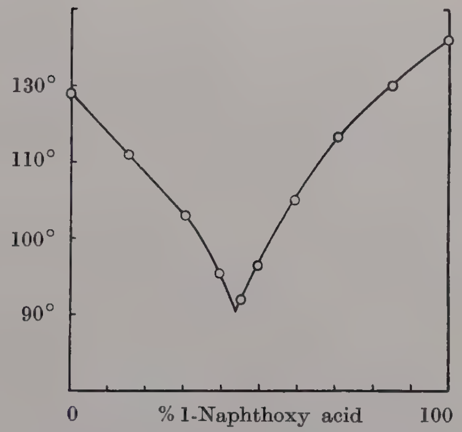
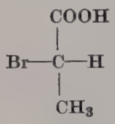


Fig. 4. (+)-α-(1-Naphthoxy)-propionic acid and (—)-α-(2-Naphthoxy)-propionic acid.

At last, we have tried to synthesize the two active naphthoxy-propionic acids from the corresponding sodium naphtholates and optically active bromopropionic acid. As the preparation of the pure antipodes of bromopropionic acid is very tedious, a partially resolved, *levorotatory* acid was used. In this way, *levorotatory* α-(1-naphthoxy)-propionic and *dextrorotatory* α-(2-naphthoxy)-propionic acid were obtained, and the activity of the products indicated that no significant racemisation had taken place. As the reactions had been performed under strictly analogous conditions, it is most unlikely that the steric course of the reaction had been different in the two cases.

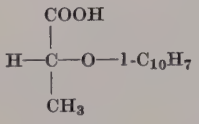
As all methods, that permit any conclusions, point to the result that the naphthoxy-propionic acids with different directions of rotation have the same configuration of the side chain, the sterical relations of these acids can be considered as definitely established.

According to FREUDENBERG (7) the (—)-bromopropionic acid should be assigned the stereoformula III. Independent work by FREDGA (5) on the halosuccinic acids supports this opinion. According to modern theories, the reaction between naphtholate ion and bromopropionate ion should be accompanied by inversion of the configuration. The (—)-α-(1-naphthoxy)-propionic acid and the (+)-α-(2 naphthoxy)-propionic acid are thus probably represented by the stereoformulas IV and V.



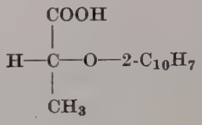
$$\begin{array}{c} \text{COOH} \\ | \\ \text{Br—C—H} \\ | \\ \text{CH}_3 \end{array}$$

(III)



$$\begin{array}{c} \text{COOH} \\ | \\ \text{H—C—O—1-C}_{10}\text{H}_7 \\ | \\ \text{CH}_3 \end{array}$$

(IV)



$$\begin{array}{c} \text{COOH} \\ | \\ \text{H—C—O—2-C}_{10}\text{H}_7 \\ | \\ \text{CH}_3 \end{array}$$

(V)

## Experimental

The *racemic acids* were prepared according to BISCHOFF (8) by heating the solid sodium naphtholates with ethyl bromopropionate and subsequent hydrolysis of the ester. Later, a more convenient method was worked out (see below).

The *racemic α-(l-naphthoxy)-propionic acid* for the melting point diagrams was recrystallised four times from benzene, once from formic acid and once from carbon tetrachloride.

0.3253 g acid: 14.11 ml 0.1064-*N* NaOH.
Equiv. wt. calc. 216.2 found 216.7

The *racemic α-(2-naphthoxy)-propionic acid* for the melting point diagrams was recrystallised once from benzene and three times from carbon tetrachloride.

0.2962 g acid: 12.86 ml 0.1064-*N* NaOH.
Equiv. wt. calc. 216.2 found 216.5

The racemic acids were resolved by the method of FOURNEAU and BALACEANO (4) and the active acids were recrystallised from benzene and carbon tetrachloride until the melting point and the optical rotation were constant. For the activity of the 1-naphthoxy-propionic acid we obtained somewhat higher values than FOURNEAU and BALACEANO, while our data for the 2-naphthoxy-propionic acid are in close agreement with the statements of these authors.

*(+)-α-(1-Naphthoxy)-propionic acid:*
0.2673 g acid: 11.61 ml 0.1064-*N* NaOH.
Equiv. wt. calc. 216.2 found 216.4

0.2380 g dissolved in abs. *alcohol* to 10.00 ml: $2\alpha = +2.36^\circ$. $[\alpha]_D^{20} = +49.6^\circ$; $[M]_D^{20} = +107.2^\circ$. — 0.1635 g dissolved in *acetone* to 10.00 ml: $2\alpha = +1.545^\circ$. $[\alpha]_D^{20} = +47.2^\circ$; $[M]_D^{20} = +102.2^\circ$. — 0.1730 g dissolved in *acetic acid* to 10.00 ml: $2\alpha = +2.53^\circ$. $[\alpha]_D^{20} = +73.1^\circ$; $[M]_D^{20} = +158.1^\circ$. — 0.1578 g dissolved in *chloroform* to 10.00 ml: $2\alpha = +1.855^\circ$. $[\alpha]_D^{20} = +58.8^\circ$; $[M]_D^{20} = +127.1^\circ$. — 0.1574 g dissolved in *benzene* to 10.00 ml; $2\alpha = +4.71^\circ$. $[\alpha]_D^{20} = +149.6^\circ$; $[M]_D^{20} = +323.5^\circ$. — 0.1591 g neutralised with *aqueous ammonia* and made up to 10.00 ml: $2\alpha = +3.05^\circ$; $[\alpha]_D^{20} = +95.9^\circ$; $[M]_D^{20} = +207.2^\circ$.

*(—)-α-(1-Naphthoxy)-propionic acid:*

0.2287 g acid: 9.92 ml 0.1064-*N* NaOH.

Equiv. wt. calc. 216.2 found 216.7

0.2265 g dissolved in abs. *alcohol* to 10.00 ml: $2\alpha = -2.24^\circ$. $[\alpha]_D^{20} = -49.4^\circ$; $[M]_D^{20} = -106.9^\circ$.

*(+)-α-(2-Naphthoxy)-propionic acid:*

0.2668 g acid: 11.62 ml 0.1064-*N* NaOH.

Equiv. wt. calc. 216.2 found 215.8

0.2102 g dissolved in abs. *alcohol* to 10.00 ml: $2\alpha = +3.925^\circ$. $[\alpha]_D^{20} = +93.4^\circ$; $[M]_D^{20} = +201.9^\circ$.

*(—)-α-(2-Naphthoxy)-propionic acid:*

0.2528 g acid: 11.01 ml 0.1064-*N* NaOH.

Equiv. wt. calc. 216.2 found 215.8

0.2122 g dissolved in abs. *alcohol* to 10.00 ml: $2\alpha = -3.96^\circ$. $[\alpha]_D^{20} = -93.3^\circ$; $[M]_D^{20} = -201.7^\circ$. — 0.1565 g dissolved in *acetone* to 10.00 ml: $2\alpha = -3.405^\circ$. $[\alpha]_D^{20} = -108.8^\circ$; $[M]_D^{20} = -235.2^\circ$. — 0.1490 g dissolved in *acetic acid* to 10.00 ml: $2\alpha = -3.55^\circ$. $[\alpha]_D^{20} = -119.1^\circ$; $[M]_D^{20} = -257.6^\circ$. — 0.1537 g dissolved i *chloroform* to 10.00 ml: $2\alpha = -1.91^\circ$. $[\alpha]_D^{20} = -62.1^\circ$; $[M]_D^{20} = -134.3^\circ$. — 0.1578 g dissolved in *benzene* to 10.00 ml: $2\alpha = -2.975^\circ$. $[\alpha]_D^{20} = -94.3^\circ$; $[M]_D^{20} = -203.8^\circ$. — 0.1540 g neutralised with *aqueous ammonia* and made up to 10.00 ml: $2\alpha = -2.26^\circ$. $[\alpha]_D^{20} = -73.4^\circ$; $[M]_D^{20} = -158.6^\circ$.

*Melting point diagrams.* The samples were prepared either by melting together weighed quantities of the substances (diagrams 1 and 2) or by dissolving the weighed quantities in pure ether (diagrams 3 and 4). In the latter cases, the ether was evaporated, and the residue was powdered and carefully dried. All melting points were determined with a Kofler hot stage microscope (6). The results are given in tables 2–5 and figures 1–4.

By studying the different substances in the hot stage microscope, three crystal modifications of the racemic α-(2-naphthoxy)-propionic acid could be detected; the melting points were 108.5°, 110° and 114°. The phase diagrams of the first and the last modifications could be studied, but the modification with m.p. 110° was very unstable. The (+)-α-(2-naphthoxy)-propionic acid had also a very unstable modification, melting at 92°.

The existence of the quasi-racemic compound was easily revealed from a study of a contact preparation in the microscope.

*Synthesis of active naphthoxy-propionic acids from active bromopropionic acid.* The (—)-bromopropionic acid, which was prepared by the method of Ramberg (9, 10), had a rotation of $\alpha_D^{20} = -27.49^\circ$ (1 dm, homogeneous). According to Timmermans and Jaffe (11), the activity of the optically pure acid is under identical conditions —50.05°. Our preparation thus contained 54.9 % of active acid.

As the method of Bischoff (8) could be expected to cause racemisation of the optically active acid, we have tried to carry out the synthesis under less drastic conditions. It was found that the naphthoxy-propionic acids could be prepared in very good yield from sodium naphtholate and sodium bromopropionate in dilute alcohol with excess of naphthol at room temperature. This method is far more convenient than that of Bischoff.

*Table 2*

(+)- and (−)-I

| % (+)-I | m.p. |
|---|---|
| 50.0 | 153.5° |
| 55.0 | 153° |
| 60.2 | 152.5° |
| 69.7 | 150° |
| 79.4 | 144° |
| 84.6 | 139.5° |
| 88.5 | 134° |
| 92.3 | 128° |
| 95.0 | 124.5° |
| 97.4 | 125.5° |
| 100.0 | 126° |

*Table 3*

(+)- and (−)-II

| % (−)-II | m.p. | |
|---|---|---|
| 50.0 | 108.5° | 114° |
| 54.9 | 108° | 113.5° |
| 59.5 | — | 113° |
| 65.0 | 106.5° | 112° |
| 70.0 | 105° | 110.5° |
| 74.7 | 108° | |
| 79.8 | 107° | |
| 85.5 | 111° | |
| 90.4 | 115° | |
| 100.0 | 119° | |

*Table 4*

(−)-I and (−)-II

| % (−)-I | m.p. |
|---|---|
| 0.0 | 119° |
| 15.1 | 111.5° |
| 24.4 | 105.5° |
| 30.2 | 102.5° |
| 34.3 | 99.5° |
| 38.3 | 98.5° |
| 41.2 | 99.5° |
| 45.0 | 100.5° |
| 49.4 | 101° |
| 54.3 | 100° |
| 58.6 | 102° |
| 64.9 | 106° |
| 70.1 | 109° |
| 84.6 | 118° |
| 100.0 | 126° |

*Table 5*

(+)-I and (−)-II

| % (+)-I | m.p. |
|---|---|
| 0.0 | 119° |
| 15.1 | 111° |
| 30.1 | 103° |
| 39.7 | 95.5° |
| 45.1 | 92° |
| 49.4 | 96.5° |
| 59.2 | 105° |
| 70.6 | 113.5° |
| 84.8 | 120° |
| 100.0 | 126° |

9.6 g (0.066 mole) of 1-naphthol and 3.0 g (0.075 mole) of sodium hydroxide were dissolved in a mixture of 15 ml water and 30 ml alcohol, and 5.2 g (0.033 mole) of active bromopropionic acid, dissolved in 15 ml alcohol were added. The mixture thus contained 0.033 mole bromopropionate, 0.042 mole naphtholate and 0.024 mole naphthol. After standing for 40 hours, the solution was evaporated to dryness at room temperature by a vigorous current af air. The residue was transferred to a separating funnel containing water and ether, and the pH was adjusted to 8 by adding dilute hydrochloric acid and ammonia. The naphthol was taken up in the ether and the aqueous layer was separated and treated with a current of air to remove the dissolved ether. On acidification with hydrochloric acid, the 1-naphthoxy-propionic acid was precipitated as a reddish-brown, immediately crystallising oil. The yield of crude product was 6.6 g (92 %).

The coloured impurities could be removed by repeated recrystallisations from benzene. As the active acid is far more soluble than the racemic compound, it was necessary to recover it from the mother liquors. Three fractions of colourless acid were obtained.

Fraction 1. 1.37 g. $[\alpha]_D^{20} = -16.2°$ (alcohol). Equiv. wt. 218.7
Fraction 2. 1.57 g. $[\alpha]_D^{20} = -37.9°$ (alcohol)
Fraction 3. 0.33 g. Practically pure racemic acid.

The two first fractions contained 32.8 and 76.6 % of active acid. The total yield of recrystallised acid was 3.27 g and its average percentage of active acid was 50.4 %

The active 2-naphthoxy-propionic acid was prepared using exactly analogous conditions from 9.2 g 2-naphthol, 5.2 g active bromopropionic acid and 3.0 g sodium hydroxide. The yield of crude product was 6.2 g (86 %). Extraction of the aqueous mother liquor with ether yielded a small quantity of bromopropionic acid.

The reddish-brown product was recrystallised several times from carbon tetrachloride. Two colourless fractions were obtained.

Fraction 1. 3.45 g. $[\alpha]_D^{20} = +35.7°$ (alcohol). Equiv. wt. 217.4
Fraction 2. 1.22 g. $[\alpha]_D^{20} = +78.2°$ (alcohol).

The two fractions contained 38.2 and 83.8 % of active acid. The total yield of recrystallised acid was 4.67 g and its average percentage of active acid was 50.1 %.

The activity data for the two acids may indicate a slight racemisation during the reaction. The mother liquors must, however, have contained an excess of the more soluble active acids, and it is more probable that practically no racemisation had taken place.

The authors are indebted to Dr. O. SNELLMAN for resolving the racemic 1-naphthoxy-propionic acid and to Messrs I. ALBERTSSON, H. BOMAN, G. BRODIN and O. ÖHRN for assistance in the synthetic work.

## SUMMARY

It was found, that α-(1-naphthoxy)-propionic acid and α-(2-naphthoxy)-propionic acid with *opposite* directions of rotation have the *same* configuration. This fact was established by the formation of a quasi-racemic compound from the acids with the same direction of rotation and by synthetic experiments, starting from optically active bromopropionic acid. No conclusions could be drawn from optical activity measurements.

A correlation has been established between the steric configuration of the side chain and the growth-regulating activity.

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REFERENCES: 1. **B. Åberg,** Arkiv Kemi. *4,* in the press. — 2. **R. L. Wain,** J. Sci. Food Agric. *2,* 101 (1951). — 3. **Chr. Winther,** Ber. *28,* 3000 (1895). — 4. **E. Fourneau** and **Balaceano,** Bull. soc. chim. France [4] *37,* 1602 (1925). — 5. **A. Fredga,** The Svedberg 1884 $\frac{30}{8}$ 1944, Uppsala 1944, p. 261. — 6. **L. Kofler** and **A. Kofler,** Mikro-Methoden zur Kennzeichnung organischer Stoffe und Stoffgemische, Innsbruck 1948. — 7. **K. Freudenberg,** Stereochemie, Leipzig and Wien 1933, p. 703. — 8. **C. A. Bischoff,** Ber. *33,* 1386 (1900). — 9. **L. Ramberg,** Ann. *349,* 324 (1906). — 10. ———, Ann. *370,* 234 (1909). — 11. **J. Timmermans** and **J. Jaffe,** Bull. soc. chim. Belg. *46,* 473 (1937).

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# Enzyme-substrate compounds and activity-pH-curves of the amylases

## I. Theoretical considerations

By Karl Myrbäck

With 2 figures in the text

Michaelis and Davidsohn (1) in 1911 advanced the theory, that the activity-pH-curves of enzymes are determined by the amphoteric properties of the enzymes. Nothing was known, at the time, about the chemical nature of enzymes, but since we, at the present time, are able to state with practically absolute certainty that all enzymes are proteins, (or contain a protein moiety) it appears possible to predict that the activity of enzymes must, a priori, be dependent on the electrical charge of the enzyme molecules.

Taking the enzyme saccharase (invertase) as an example, the theory of Michaelis and Davidsohn, in its simplest form, implies that only uncharged enzyme molecules possess enzymatic activity, whereas the ions are inactive. In other words, it is assumed that the activity optimum of the enzyme coincides with its isoelectric point. The question why enzymatic activity should be displayed only by uncharged enzyme molecules can certainly not be answered at the present time. Moelwyn-Hughes (2) has called attention to the fact that charged enzyme molecules are liable to attract water dipols to their surfaces, thereby possibly making the contact between enzyme and substrate more difficult. The same author also points out that an uncharged enzyme molecule may very well possess charged groups provided that the numbers of positive and negative charges or groups are equal.

Michaelis and Menten (3) were the first to express clearly the opinion that the enzyme action on a substrate is mediated by an enzyme-substrate compound the concentration of which is determined by the mass action law. There is no reliable evidence that an enzyme could act on its substrate by long range forces; the concept of the enzyme-substrate compound as mediator of the enzyme action is fundamental to enzyme chemistry.

If an enzyme-substrate combination product is the molecule in which the enzymatic reaction takes place, it seems clear that the Michaelis-Davidsohn theory of the uncharged enzyme molecules as the only active ones can at least not have general applicability. Take for instance, the enzyme trypsin acting on an ordinary protein at $pH = 8$. At this pH the substrate would be electrically charged and it seems hardly probable that the uncharged enzyme molecules should combine with the substrate ion. And also in those cases where the substrate, *e.g.* a disaccharide, does not alter its charge in the pH-range of enzyme action, the question arises whether the inactivity of the enzyme ions, as postulated by MICHAELIS and DAVIDSOHN, should be explained by the assumption that the enzyme ions do not combine with the substrate, or that they actually combine with the substrate but form a product, probably an ion, which does not undergo the enzymatic transformation.

MICHAELIS and ROTHSTEIN (4) tried to unite the theory of the enzyme-substrate combination and the theory of the activity-pH-curve as a dissociation curve in the following way: Whereas MICHAELIS and DAVIDSOHN and assumed that the alkaline branch of the pH-curve of saccharase is the dissociation curve of the enzyme presumed to be a weak acid with $K_a = 10^{-6.6}$, MICHAELIS and ROTHSTEIN instead assume that the enzyme-substrate compound is an acid with the same dissociation constant. However, KUHN (5) pointed out, obviously assuming as MICHAELIS and ROTHSTEIN had done that the enzyme itself does not alter its charge in the pH-range in question, that this new theory of MICHAELIS and ROTHSTEIN leads to the conclusion that the alkaline branch of the pH-curve must vary with the substrate concentration. Since this certainly is not the case, KUHN concluded that the theoretical approach of MICHAELIS is entirely wrong. KUHN assumed instead that the rate of desintegration ("Zerfallsgeschwindigkeit") of the enzyme-substrate compound varies with pH.

However, if according to the original theory of MICHAELIS and DAVIDSOHN the enzyme itself is an acid with $K_a = 10^{-6.6}$, the assumption does not seem necessary or even probable that the combination of enzyme and substrate should be mediated by the acid groups of the enzyme or even influence the acid character of these groups. If it be assumed (6) that not only the enzyme, but also the enzyme-substrate compound are acids with the same $K_a$ it is easily found (see below) that the alkaline branch of the pH-curve of the saccharase must be independent of substrate concentration, which is in accordance with the experiments.

When extending the investigation to the acid region of the pH-optimum the present author found (7) the pH-curve to depend in a quite characteristic way on the substrate concentration. When the substrate concentration is increased the acid branch of the pH-curve is displaced parallel to itself toward the acid side. This is easily understood if it be assumed that the combination of enzyme and substrate involves the basic groups of the enzyme. In other words, it is assumed that the substrate binding group of the enzyme is a basic group (or any number of basic groups) which determines the ionisation of the enzyme in acid solution. Since other experiments (inactivation by nitrous acid etc.) indicated that the group in question is a primary amino-group, it is assumed that the uncharged amino-group -$NH_2$, but not the charged ammonium grouping- $NH_3^+$, combines with the substrate. Using a modern way of speaking we might say that the reversible inactivation of the saccharase by $H^+$-ions, *i.e.* the formation of the ammonium groupings, is *competitive*. Assuming that saccharase is an ampholyte with $K_a = 10^{-6.6}$ and $K_b = 10^{-11}$ and that the MICHAELIS constant is $K_m = 0.040$ and finally that the substrate is bound by the undissociated basic groups, a very good agreement was found between theory and experiment. (The value of $K_m$ was determined in the usual way; the value may be different for saccharase of other origin and perhaps also dependent on the degree of purity of the enzyme preparation (8). The values of the constants for the pure enzyme are not known, but the assumed values are quite reasonable. Using a preparation of the activity $If = 240$ (9) MYRBÄCK found (10) by direct titration $K_b = 3.10^{-10}$; an other preparation, investigated by JOSEPHSON gave $K_b = 5.10^{-11}$.

It should be emphasized at this point that acceptance of the Michaelis theory does not necessitate the conclusion that the isoelectric point of the enzyme must coincide with the pH-optimum, even in those cases where the substrate is not an electrolyte. Evidently the circumstances must be complicated when the substrate alters its charge in the pH-range in question but, even if this is not the case, the position of the pH-optimum is, at least to some degree, dependent on the substrate concentration (see below) and, furthermore, it can safely be assumed that the enzyme molecule possesses several basic groups of varying basicity, among which perhaps only one or few are involved in the fixation of the substrate on the enzyme surface.

Evidently, in the Michaelis theory and the modifications thereof mentioned above, it is assumed that the rate of the enzymatic reaction is always directly proportional to the concentration of the uncharged enzyme-substrate compound, in which the reaction is presumed to take place, with a velocity independent of the pH of the medium. Implicitly it is also assumed that the velocity of formation of the enzyme-substrate compound is infinitely high compared to the velocity of the enzymatic reaction proper.

In the original paper by MICHAELIS and MENTEN the equilibrium

$$\text{enzyme} + \text{substrate} \rightleftarrows \text{active compound}$$

was investigated only at the pH-optimum of the enzyme. In other words, the ampholytic properties of the enzyme were disregarded. If the total enzyme concentration is $\Sigma$ and the concentration of free enzyme is [E], then

$$K_m = [S] \cdot \frac{[E]}{\Sigma - [E]}$$

where [S] is the substrate concentration. $\Sigma - [E]$ is proportional to the velocity of the reaction at a substrate concentration [S], and $\Sigma$ itself is in the same way proportional to the maximum velocity at very high substrate concentration. Ordinarily this maximum velocity can be determined easily by extrapolation. The value of $K_m$ is equal to $[S_{0.5}]$, *i.e.* to the substrate concentration at which the actual reaction velocity equals one half of the maximum velocity ($[E] = 0.5\,\Sigma$).

Now if we consider the alkaline branch of the saccharase pH-curve and, following MICHAELIS and DAVIDSOHN, regard the enzyme as a weak acid with a certain $K_a$-value we have, in the pH-range 4—9 (where the concentration of positive enzyme ions is practically zero and the dissociation of the basic groups can be disregarded), uncharged enzyme molecules, HE, and negative enzyme ions, $E^-$. If we assume that only HE is able to combine with the substrate S to give the active enzyme-substrate compound HES, then we have the following equations:

$$[HE] \cdot [S] = K_1 \cdot [HES]$$

$$[H^+] \cdot [E^-] = K_a \cdot [HE]$$

$$\Sigma = [HES] + [HE] + [E^-].$$

Hence we find

$$K_1 = [S] \cdot \frac{\Sigma - [HES]}{[HES]} \cdot \frac{1}{1 + K_a/[H^+]}.$$

If $[S_{0.5}]$ is the substrate concentration where $[HES] = 0.5\ \Sigma$ then

$$K_1 = [S] \cdot \frac{1}{1 + K_a/[H^+]}$$

*i.e.* $K_m$ is only an apparent constant and will vary with pH, or conversely, as shown by KUHN, the pH-curve will vary with the substrate concentration.

However, if we assume that not only HE but also $E^-$ combines with S to yield active molecules HES and inactive ions $^-ES$, then we have

$$([HE] + [E^-]) \cdot [S] = K_2\ ([HES] + [^-ES])$$

$$\frac{[H^+] \cdot [E^-]}{[HE]} = \frac{[H^+] \cdot [^-ES]}{[HES]} = K_a$$

$$\Sigma = [HES] + [HE] + [^-ES] + [^-E]$$

and

$$K_2 = [S] \cdot \frac{\Sigma - [HES] \cdot (1 + K_a/[H^+])}{[HES] \cdot (1 + K_a/[H^+])}$$

or

$$K_2 = [S_{0.5}]$$

where $[S_{0.5}]$ is the substrate concentration where HES $(1 + K_a/[H^+] = 0.5\ \Sigma$. In this case $K_2$ $(= K_m)$ is independent of pH and the pH-curve is independent of the substrate concentration. As already mentioned this is, in the case of saccharase, in agreement with the experiments.

We may now consider the acid branch of the pH-curve (pH = 2–5) where the dissociation of the acid group(s) of the enzyme can be disregarded. If the enzyme possesses basic groups altering their charge in the pH-range mentioned, then we have, in addition to the uncharged enzyme molecules $ENH_2$ (where $NH_2$ may symbolize any basic group) also the ions $ENH_3^+$. We may now assume, either that only $ENH_2$ or both $ENH_2$ and $ENH_3^+$ combine with the substrate to yield the active molecule $ENH_2S$ and the inactive product $ENH_3^+S$. (It is perhaps superfluous to point out that the hypothetic product $ENH_3^+S$ and the negative ion $^-ES$ can *not* be supposed to be *active*, because in this case the enzyme activity would be independent of pH). As above we find, that if both $ENH_2S$ and $ENH_3^+S$ are supposed to exist, the pH-curve must be independent of substrate concentration. On the other hand, if we assume that only the uncharged enzyme molecules $ENH_2$ are able to combine with the substrate, then we find

$$K_3 = [S_{0.5}] \frac{1}{1 + K_b/[OH^-]}$$

Just as it is found in the case of saccharase to the acid side of the pH-optimum the pH-curve will vary with the substrate concentration, or the apparent MICHAELIS constant, defined as $K_m = [S_{0.5}]$, will vary with the pH.

Evidently, if the MICHAELIS constant of an enzyme is determined as $K_m = [S_{0.5}]$ and is found to be independent of pH, it must be concluded either that the enzyme does not alter its charge in the pH-range in question or that both the uncharged enzyme molecules *and* the enzyme ions do combine with the substrate.

An apparent variation of the MICHEALIS constant with pH has been observed in certain other cases. D. D. VAN SLYKE and his collaborators, in fundamentally important investigations on enzyme action (11), found that in the case of urease experiments at pH = 6 yield an apparent value of the MICHAELIS constant, which is eleven times as great as that found at pH = 7. The authors explain this by the assumption that the *velocity of formation* of the enzyme-substrate compound (which must be proportional to the substrate concentration) varies with the pH of the solution. Since urease is a protein with several weakly acidic and basic groups, which must be presumed to alter their charge in the pH-range of urease action, the idea was submitted by the present author (12), that the assumed variation of a *velocity* with the pH is in reality a variation of the *concentration* of the reacting molecules. It could be shown that the pH-curves at different substrate concentration determined by VAN SLYKE agree astonishingly well with those calculated in the same way as the pH-curve of saccharase.

At least in the case of the enzymes mentioned above it appears well established that the electric charges of the enzyme molecules and the enzyme-substrate compounds are the factors which, at a certain temperature, determine the velocity of the enzymatic reaction. In a broad sense this was assumed already in 1911 by MICHAELIS and his collaborators, but the original theories have had to be modified and further elaborated, and it seems possible to state, at least in the case of the enzymes mentioned, that there are among the substrate-binding groups of the enzymes certain basic groups, which lose their binding capacity on transformation into charged (presumably ammonium) form. Furthermore, it can be stated that the enzymes possess weakly acid groups which have nothing to do with the binding of the substrate. These acid groups, however, determine the behaviour of the enzyme-substrate compound: If the acid groups are undissociated (HES) the enzymatic reaction takes place; if the protons are split off the remaining negative ion ($^-$ES) is stable in the sense that the enzymatic recation does not take place. Tentatively it was assumed (12) that the undissociated acid groups represent regions of high potential hydrogen ion concentration on the enzyme surface, and that the enzymatic hydrolysis of the substrate takes place because the latter is bound by the substrate-binding groups (or "binding area") in such a way that the linkage to be hydrolyzed is brought into close contact with the "acid region".

The calculation of the pH-curves of urease at different substrate concentrations was carried out under the assumption that the enzyme possesses acidic and basic groups with $K_a = 10^{-9}$ and $K_b = 5.10^{-8}$ respectively. If all acidic and basic groups of the enzyme molecule had these $K$-values, the isoelectric point of urease would be something like 7.7. However, the direct determination of the isoelectric point of crystalline urease has yielded values in the neighbourhood of 5.0. Even if this value is not absolutely accurate (it is found to vary with the buffer solution used; see below) it does not seem possible to assume that the correct value would be 7.7. However, it should be pointed out that the experimental value 5.0 for the isoelectric point does not prevent the assumption that acidic and basic groups with the dissociation constants mentioned above are present in the enzyme molecule, since the electrically neutral molecule may well contain charged groups provided that the negative and positive charges are equal in number.

VAN SLYKE and his collaborators observed that phosphate and certain other salts, even in rather low concentrations, have a retarding effect on the enzymatic hydrolysis of urea. The explanation given by them was that the salts decrease the *rate of formation* of the enzyme-substrate compound. However, the fact that the retarding effect of phosphate depends in a most characteristic way on the pH of the medium, seems to offer an other explanation of the experimental facts. To the alkaline side of the pH-optimum (pH = 7.0 at a substrate concentration = 0.15 M) the percentual inhibition by phosphate is practically independent of pH (see fig. 3 in the paper by VAN SLYKE and ZACHARIAS (11)). In acid solution, on the contrary, the percentual inhibition increases strongly with increasing acidity. In fact, increase of the phosphate concentration displaces the acid branch of the pH-curve parallel to itself toward the alkaline side. Precisely the same was found by the present author (7) when investigating the inactivation of saccharase with picric acid, phosphotungstic acid and other substances which can be presumed to act on the enzyme by forming complexes with the basic groups of the enzyme molecule. In these cases the inhibition was found to be typically competitive, strongly supporting the conclusion that the basic groups are involved in the fixation of the substrate to the enzyme molecule. It seems natural to assume that the inhibition of urease by phosphate and other ions is due also to formation of complexes between the basic groups of the enzyme and the anions.

HOWELL and SUMNER (13) investigated the dependance of urease activity upon the type of buffer, the buffer concentration and the urea concentration. In M/8 phosphate buffer the pH-curves for different substrate concentrations were found to be almost identical in alkaline solution whereas, to the acid side of the pH-optimum the curves show the same displacement with increasing substrate concentration that was observed also by VAN SLYKE and by LÖFGREN (14). Displacement of the pH-optimum toward the acid side was found also for citrate and acetate buffers. In both cases, as can easily be seen by comparison of Fig. 5 and 6 of the HOWELL-SUMNER paper, the displacement of the pH-optimum is caused by a displacement of the acid branch of the curve, whilst the alkaline branch is not or only slightly influenced by the substrate concentration. The pronounced differences in the pH-curves, which are found when different buffers are used, together with the fact already mentioned, that the isoelectric point of the crystalline enzyme varies with the buffer used, will perhaps allow the conclusion, that the various values of $K_a$ and $K_b$ mentioned above do not refer to the enzyme itself but to combinations of the enzyme with various ions.

The influence of different buffer salts on the activity of wheat β-amylase has been investigated by BALLOU and LUCK (15). They compared the pH-curves found in formate, acetate, propionate, butyrate and valerate buffers of the same ionic strength. At the pH-optimum and to the alkaline side thereof the same curve was found, but in the acid region the curve was displaced toward the alkaline side with increasing numbers of carbon atoms in the carboxylate ion. It appears probable that, also in this case, the reversible and presumably competitive inhibition of the enzyme by the ions of higher fatty acids is due to formation of not completely dissociated complexes between the basic groups of the enzyme and the carboxylate ions. A similar action was found with phenylacetate and phthalate ions. The action is probably of the same type as the very strong action exerted by higher alkyl sulfates, heparin etc. which will be described in a following paper. In these cases the inactivation is not instantaneous and is irreversible, probably because the binding of the large negative ions to the basic groups of the enzymes causes a denaturation of the enzyme protein, perhaps an unfolding of the polypeptide chains.

## II. pH-curves of a mold amylase

By KARL MYRBÄCK and EBBA WILLSTAEDT

In the following some experiments will be described which were carried out with a mold amylase in order to determine whether the pH-curve shows a dependance on the substrate concentration similar to that observed with saccharase and urease. The enzyme was prepared from "Diastase Merck, Ph. Jap. IV". The enzyme is a typical α-amylase. A weak maltase action of the original preparation was abolished on storing or gently heating of the solution.

In order to avoid precipitation of starch components during the experiments and difficulties in the sugar determinations etc. a Lintner starch was used as substrate. This starch was prepared in the usual way by treating potato starch with cold 2 % hydrochloric acid for 30 days and thorough washing. A 4.40 % starch solution was used throughout the experiments.

Mixtures of 0.3 mol phosphoric acid with varying amounts of sodium hydroxide were used as buffer. pH was determined electrometrically in the reaction mixture of each experiment. The mixtures of starch solution, buffer and water were brought to 30° in a water thermostate. The enzyme solution, heated to the same temperature, was added and samples were removed after suitable times for sugar determination. The WILLSTÄTTER-SCHUDEL method was used and the reduction values calculated as mg glucose. Four to six sugar determinations were made in each experiment. The increase in reduction value with time showed that no inactivation of the enzyme took place during the experiments, even in the most acid solutions. Two series of experiments were performed, one at a starch concentration of 2.20 %, the other at 0.22 %. One experiment of each series will be described in some detail:

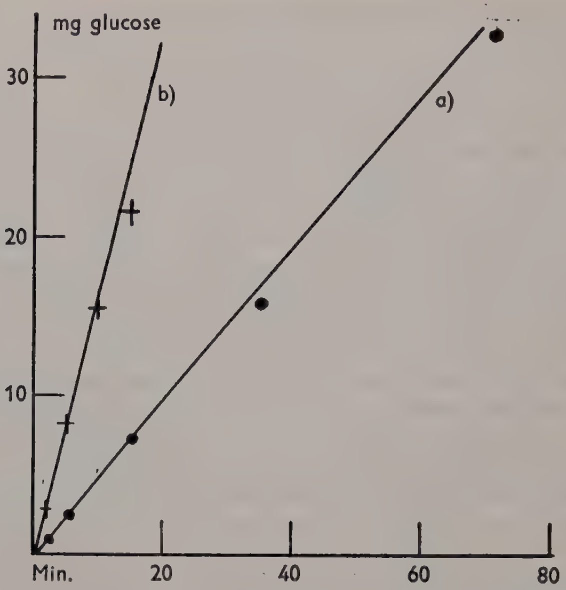

Fig. 1. Increase of reducing power in experiments a) and b).

a). 25 ml starch solution, 5 ml phosphate buffer, 15 ml water, 5 ml enzyme solution; pH = 4.02. Sugar determinations in 5 ml samples; table. 1. The curve, showing the liberation of reducing groups (Fig. 1) is practically a straight line. Therefore, we may use, for instance, the value "mg glucose/minute" as expressing the reaction velocity. The curve shows this value to be 0.50 mg.

b). 25 ml starch solution, 50 ml phosphate buffer, 400 ml water, 25 ml enzyme solution; pH = 7.47. Sugar determinations in 50 ml samples; table 1;

*Table 1*

| Experiment a) | | Experiment b) | |
|---|---|---|---|
| Minutes | mg glucose | Minutes | mg glucose |
| 2 | 1.14 | 2 | 2.74 |
| 5 | 2.71 | 5 | 8.04 |
| 15 | 7.28 | 10 | 15.50 |
| 35 | 16.06 | 15 | 21.74 |
| 70 | 32.73 | | |

The curve (Fig. 1) representing experiment b) is very slightly curved. The initial velocity is 1.57 mg glucose/minute. Since the enzyme comcentration in series b) is only one half of that in series a) but the titrated volume is ten times as large, we have to multiply the velocity values of series b) with 2/10 to get values comparable to those of series a). Therefore, the velocity of experiment b) is 0.31 mg glucose/minute, if expressed in the same units as in series a).

The reaction velocities, calculated as above, at different pH's can be found in table 2.

*Table 2*

| 2.20 % starch | | 0.22 % starch | |
|---|---|---|---|
| pH | mg glucose/min. | pH | mg glucose/min. |
| 3.02 | 0.29 | 2.96 | 0.13 |
| 3.30 | 0.33 | 2.98 | 0.13 |
| 3.44 | 0.43 | 3.20 | 0.21 |
| 3.79 | 0.48 | 3.97 | 0.38 |
| 4.02 | 0.50 | 4.62 | 0.46 |
| 4.35 | 0.52 | 4.63 | 0.48 |
| 5.32 | 0.57 | 6.61 | 0.48 |
| 5.74 | 0.54 | 7.47 | 0.31 |
| 6.17 | 0.57 | 7.90 | 0.20 |
| 6.50 | 0.54 | | |
| 6.97 | 0.46 | | |
| 7.31 | 0.43 | | |
| 7.92 | 0.18 | | |
| 8.2 | ~0.05 | | |

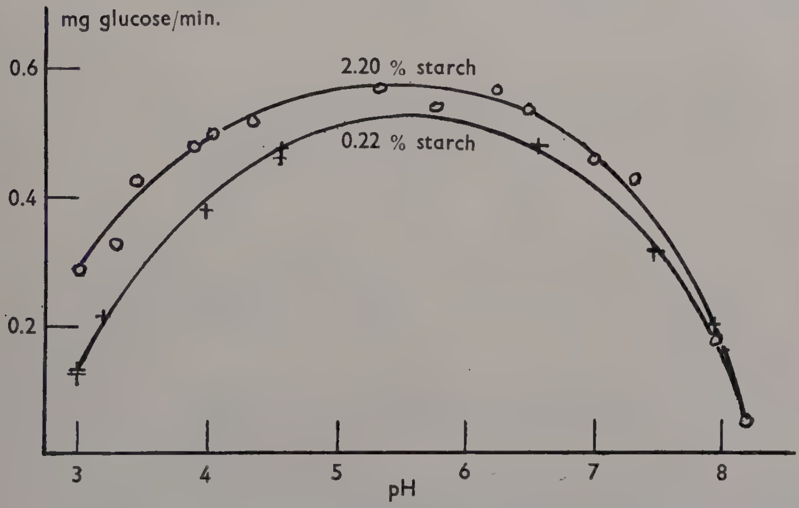

Fig. 2. pH-curves at two different substrate concentrations.

The two pH-curves are plotted in Fig. 2. It is easily seen that, just as in the case of saccharase and urease, the alkaline branch of the activity-pH-curve is independent of the substrate concentration whereas the acid branch is displaced toward the acid side with increasing substrate concentration. It seems therefore possible to apply the same theory of the enzyme-substrate compound here, namely that basic groups in the uncharged state bind the substrate whereas the same groups in the ionic state do not, and that the acid groups of the enzyme do not bind substrate but are, in the uncharged form, indispensable to the enzymatic activity.

REFERENCES. 1) **Michaelis, L.** and **Davidsohn, H.,** Biochem. Z. *35,* 386 (1911). — 2) **Moelwyn-Hughes, E. A.,** *Physical chemistry and chemical kinetics of enzymes,* in J. B. Sumner and K. Myrbäck: *The Enzymes, Chemistry and Mechanism of Action,* Vol. I, Academic Press, New York 1950. (p. 28). — 3) **Michaelis, L.** and **Menten, L.,** Biochem. Z. *49,* 333 (1913). — 4) **Michaelis, L.** and **Rothstein, M.,** Biochem. Z. *110,* 217 (1920). — 5) **Kuhn, R.,** Z. physiol. Chem. *152,* 28 (1923). — 6) **v. Euler, H., Josephson, K.** and **Myrbäck, K.,** Z. physiol. Chem. *134,* 39 (1924). — 7) **Myrbäck, K.,** Z. physiol. Chem. *158,* 160 (1926). — 8) **Sumner, J. B.** and **Myrbäck, K.,** *The Enzymes,* p. 16 (see reference 2). — 9) **v. Euler, H.** and **Josephson, K.,** Ber. *56,* 1749 (1923). — 10) **Myrbäck, K.,** Arkiv kemi, mineral. geol. *9,* Nr 2 (1923). — 11) **van Slyke, D. D.,** Advances in enzymology. *2,* 33 (1942); **van Slyke, D. D.** and **Cullen, G. E.,** J. Biol. Chem. *19,* 141 (1914); **van Slyke, D. D.** and **Zacharias, G.,** J. Biol. Chem. *19,* 181 (1914). — 12) **Myrbäck, K.,** Acta Chem. Skand. *1,* 142 (1947). — 13) **Howell, S. F.** and **Sumner, J. B.,** J. Biol. Chem. *104,* 619 (1934). — 14) **Lövgren, S.,** Biochem Z. *119,* 215 (1921); *137,* 206 (1923). — 15) **Ballou, G. A.** and **Luck, J. M.,** J. Biol. Chem. 139, 233 (1941).

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# Enzymchemische Studien an Blättern von Espen und an Keimblättern von Gerste. I

Von Hans von Euler und Armin Glaser

## A. Untersuchungen von Blättern diploider und triploider Espen

Die hier mitzuteilenden Bestimmungen sind an einem Material ausgeführt, welches sich aus röntgenbestrahlten sowie unbestrahlten Pollen diploider und tetraploider Espen entwickelt hatte.

Das Ziel einer 1944 mit H. Nilsson-Ehle begonnenen Untersuchung war teils der chemische Vergleich zwischen gewöhnlichen, diploiden Espen und den triploiden (3 × 19 = 57) Gigas-Formen, teils das Studium der mit Mutationen verknüpften chemischen, besonders enzymchemischen Veränderungen in den untersuchten Pflanzen.

Das mit Fil. Lic. Carin Ehrenberg hergestellte Ausgangsmaterial ist in einer vorhergehenden Arbeit[1] kurz beschrieben.[2] Cytologische Untersuchungen der Wurzeln unserer Pflanzen sind in verschiedenen Jahren von C. Ehrenberg ausgeführt worden und werden später zusammengestellt.

Die vorliegende Mitteilung bezieht sich besonders auf die *Blätter* unserer Pflanzen.

Orientierende chemische Versuche an Wurzeln, besonders an Wurzelspitzen, die — so lange nicht Pollen der nächsten Generation zur Verfügung stehen — a priori am aussichtsreichsten erscheinen — stossen auf verschiedene experimentelle Hinderungen.

Besonders zu erwähnen ist hierbei die Schwierigkeit, die Wurzelspitzen zu wiederholten Bestimmungen in ausreichenden Mengen zu beschaffen und die zu enzymchemischen Untersuchungen notwendige Homogenisierung ohne störende Inaktivierung durchzuführen.

Wenn wir uns also zunächst auf die Untersuchung der *Blätter* konzentriert haben, obwohl die mannigfache Abhängigkeit der Entwicklung und des Stoffwechsels der Blätter von Umweltbedingungen die Erkennung von Mutationen, überhaupt von genetisch bedingten Veränderungen erschwert, so geschah dieses in Anbetracht ihres relativ hohen Gehaltes an zahlreichen Enzymen, Wuchsstoffen, Hormonen und Hemmungsstoffen, und von der Voraussetzung ausgehend, dass sich hier zeitig mutative Veränderungen zu erkennen geben. Trotz der Umweltbedingtheit der Blattentwicklung sind nicht wenig Fälle bekannt, in welchen — wie z. B. bei *Gossypium arboreum* (HAMMOND, Am. J. of Bot. 1946) der Zusammenhang der Blattform von definierten Genen einwandfrei gezeigt werden konnte.

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[1] Euler, Dieses Arkiv 26 A No 30 (1948).

[2] Die im Herbst 1944 auf die beiden Versuchsfelder Solna und Haga (Stockholms Norra Begravningsplats) verteilten Pflanzen (ca 5000) erhielten in den Jahren 1946 und 1947/48 durch Umpflanzungen ein grösseres Areal, nämlich 1,5 × 1 m per Espe.

Über die *Grössen und Formen* der Blätter wurden in der vorhergehenden Arbeit schon einige Angaben gemacht, unter Hinweis auf die von Abbildungen begleiteten Äusserungen von NILSSON-EHLE und von I. BERGSTRÖM über die Vergrösserung der Blätter bei tetraploiden Espen.

Wie der Verf. schon früher betont hat, liegen bezgl. der Grössenverteilung der Blätter eines Individuums ferner bezgl. Abhängigkeit dieser Verteilung von Alter, Entwicklungsstadium, Jahreszeit, Temperatur, Belichtung, Bodenbeschaffenheit und Ernährungsbedingungen Probleme vor, die noch wenig bearbeitet worden sind.

Soweit diese Probleme die Abhängigkeit *morphologischer Eigenschaften* der Blätter von ständigen (Bodenbeschaffenheit) oder rhythmisch variierenden (meteorologischen) Umweltfaktoren betreffen, besitzen wir keine systematisch gesammelten Versuchsdaten. Dagegen hing es direkt mit unserer Aufgabe zusammen, eventuelle Beziehungen zwischen enzymatischen Wirksamkeiten und Blattgrösse mit in Betracht zu ziehen, und wir haben uns hier darauf beschränkt, solche Beziehungen aufzusuchen.

Im Zusammenhang mit den enzymchemischen Untersuchungen über die Verschiedenheiten der Blätter eines und desselben Individuums, war es notwendig, wenn auch nicht die Form[1] so doch die *Grösse* der untersuchten Blätter zu beobachten und mit ihrem Wuchsstoff-Gehalt zu vergleichen.

Es kommen da zunächst die grossen Differenzen in Betracht, welche bei einem Individuum (etwa einer 3 m hohen Espe) bezgl. der Stellung (untere Zweige oder Gipfel) und bezgl. der Jahreszeit bestehen. Solche Bestimmungen wurden bisher an 20 gleichaltrigen Espen vorgenommen.

Ferner wurden unter möglichst vergleichbaren Umständen 11 Espen während 4 Jahren (1947–1950) je 2 mal, nämlich im Mai und im August, vergleichend untersucht. Regelmässigkeiten im Einfluss der Jahresvariationen konnten wir noch nicht feststellen. Exakte Messungen über jährliche Veränderungen der Blattgrössen, wie sie etwa bzgl. des Wachstums von Bäumen von F. F. BLACKMAN ausgeführt worden sind, liegen noch nicht vor.

Die metereologischen Enflüsse[2] (Temperatur, Feuchtigkeit) sind offenbar hier viel grösser als der jahresrhythmische Einfluss kosmischer Strahlungen. Ein Einfluss der Tagesrhythmen, wie er sich auf die Spaltöffnungen zeigt, hat sich an den Blättern der 5–6 Jahre alten Espen hinsichtlich des Enzymgehaltes nicht feststellen lassen.

Zur Erleichterung der Übersicht über die folgenden Angaben sei nochmals[3] die Liste der untersuchten Kreuzungen mit ihren Bezeichnungen vorangestellt (N.-E. tetrapl. ♂ = NILSSON-EHLES tetraploide ♂ Espen).

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[1] Eine orientierende Studie über die Blattformen, die bald an anderer Stelle mitgeteilt werden soll, ergab bemerkenswerte Resultate über die Beeinflussung der Stellung, Form u. Zähnung von Espen- u. a. Blättern durch verschiedene chemische Ergone.

[2] F. W. WENT, Science 112, Oct. 27; 1950.

[3] EULER, Dieses Arkiv. 26 A No 30 (1948/49).

| | |
|---|---|
| Ser. I. | Ekebo diploid ♀ × N.-E.s tetrapl. ♂ unbestrahlt |
| Ser. II. | Ekebo dipl. ♀ × N.-E.s tetrapl. ♂ bestrahlt mit 1000 r. Pollen ges. 2 Tage nach Bestrahlung |
| Ser. III. | Ekebo dipl. ♀ × N.-E.s tetrapl. ♂ bestrahlt mit 1000 r. Pollen ges. 3 Tage nach Bestrahlung |
| Ser. IV. | Unbestrahlte Kontrolle: Ekebo dipl. ♀ × Ekebo dipl. ♂ |
| Ser. V. | Ekebo dipl. ♀ × Ekebo dipl. ♂ bestrahlt 1000 r. Pollen ges. 3 Tage später |
| Ser. VI. | Ekebo dipl. ♀ × Ekebo dipl. ♂ bestrahlt 1000 r. Pollen ges. 4 Tage später |
| Ser. VII. | Ekebo dipl. ♀ × Ekebo dipl. ♂ bestrahlt 500 r. Pollen ges. 3 Tage später |
| Ser. VIII. | Ekebo dipl. ♀ × Ekebo dipl. ♂ bestrahlt 500 r. Pollen ges. 4 Tage später |
| Ser. IX. | Ekebo dipl. ♀ × Ekebo dipl. ♂ bestrahlt 10000 r. Keine überleb. Pfl. |
| Ser. X. | Ekebo dipl. ♀ bestrahlt 1000 r. × Ekebo dipl. ♂ Pollinier. 3 Tage später |
| Ser. XI. | Ekebo dipl. ♀ × Ekebo dipl. ♂ bestrahlt 10000 r. Pollen ges. am Tag der Bestrahlung und am folgenden Tag |
| Ser. XII. | Ekebo dipl. ♀ × Ekebo dipl. ♂ unbestrahlte Kontrolle. |

## Gehalt der Espenblätter an Enzymen. Enzymatische Aktivitäten

Wie in unserer vorhergehenden Mitteilung bezieht sich die Mehrzahl der 1949 und 1950 ausgeführten enzymatischen Bestimmungen auf Katalase. Auch über die Aktivität von Dehydrasen in Espenblättern haben wir (l. c. S. 14) bereits einige Angaben gemacht, u. zw. über Succino-Dehydrase und Isocitroco-Dehydrase[1]; dieselben werden ergänzt. Von den übrigen enzymatischen Untersuchungen, die nun auf Peroxydase, Phenol-Oxydasen, Carboxylase, Amylase, Trans-Aminasen und Phosphatasen ausgedehnt wurden, seien hier diejenigen über Peroxydase und Phosphatase angeführt.

Der Gehalt bezw. die Wirksamkeit an Amylase und Transaminase erwiesen sich so gering, dass die Fehlergrenzen der Bestimmungen zu gross waren.

## Katalase

Bezüglich der titrimetrischen Versuchsmethodik kann auf zahlreiche frühere Arbeiten aus diesem Institut verwiesen werden.[1] Die Extraktion wurde unter Zusatz von Sand ausgeführt. Besonders sei hervorgehoben, dass die Dauer und die Intensität des Verreibens der Blätter (es wurden immer 4—5 gleichartige Blätter gemeinsam verrieben) die Katalase-Aktivität stark beeinflusst. Dieser Umstand kann erhebliche methodische Fehler veranlassen, und man muss also für jedes Material die Bedingungen des Zerreibens aufsuchen, unter welchen die maximalen Aktivitätswerte erhalten werden. Unter Einhaltung der geeigneten Vorsichsmaassregeln (Titration bei 0°; $H_2O_2$-Konzentration nicht über 0,01 normal) ist es dann möglich, vergleichbare Aktivitätswerte zu erhalten.

Auch hinsichtlich der Berechnung unserer Versuche können wir auf die früheren einschlägigen Arbeiten dieses Institutes verweisen.[2]

Für eine im September 1950 ausgeführte Versuchsreihe geben wir die Premiärwerte an, welche über die Fehlergrenzen und die Variationen innerhalb einer solchen Serie Auskunft gibt. In Tab. 3 sind die kat.f-Werte, also die auf die Trockengewichte bezogenen Reaktionskonstanten, zusammengestellt.

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[1] EULER, E. ADLER, G. GÜNTHER u. L. ELLIOT Enzymologia 6, 337 (1939).

[2] EULER u. J. v. EULER, Dieses Arkiv 26 A No 22 (1948/49).

Tab. 2 gibt ein Beispiel für die *grossen Unterschiede der Katalasewirkung der an einem und demselben Tag* gepflückten Blätter eines Baumes.

Die Blätter wurden mit einer Phosphat-Pufferlösung von pH = 6,8 extrahiert von diesem Extrakt wurden für jede Bestimmung 3 ml abpippettiert und mit 10 ml einer 0,01 n. $H_2O_2$-Lös. versetzt. Temp. 0°. Abbruch durch Zusatz von Schwefelsäure.

Es sei auch hier darauf aufmerksam gemacht, dass wir aus den Aktivitäts-Bestimmungen nicht den Katalase-Gehalt, sondern die Katalase-*Wirkung* berechnen. Die Aktivität ist durch noch unbekannte Aktivatoren und Hemmungs-Stoffe (Inhibitoren) der Lyo- und Desmo-Katalase beeinflusst.

*Tab. 1.*

*Espe V 37 Blatt-Grösse 5,2 × 4,7 cm²*. — Bestimmungen vom 20.9.50.

Trockengewicht: 186,5 mg frisches Blatt ergaben 83,0 mg trocken.
Einwaage: 101,5 mg.

| Min. | $a$ | $a-x$ | $k$ | kat.f = $k/g$ Trockensubst. | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 8,6 | 0,01186 | 1,7 | |
| 16 | — | 7,5 | 0,00964 | 1,4 | 1,5 |
| 24 | — | 6,4 | 0,00930 | 1,3 | |

Eigenverbrauch: 1,00.

*Espe V 35 Blatt-Grösse 4,15 × 4,5 cm²*

Trockengewicht: 161,0 mg frisches Blatt ergaben 79,2 mg trocken.
Einwaage: 125,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 7,8 | 0,01716 | 1,8 | |
| 16 | — | 6,7 | 0,0127 | 1,3 | 1,4 |
| 24 | — | 5,9 | 0,0107 | 1,1 | |

Eigenverbrauch 1,3.

*Espe I 128 Blatt-Grösse 5,8 × 5,3 cm²*

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 9,2 | 0,0082 | 1,04 | |
| 16 | — | 8,1 | 0,0075 | 0,94 | 0,95 |
| 24 | — | 7,3 | 0,0069 | 0,87 | |

Eigenverbrauch 0,9.

*Espe I 190 Blatt-Grösse 5,3 × 5,5 cm²*

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 9,8 | 0,00476 | 0,53 | |
| 16 | — | 9,5 | 0,00323 | 0,36 | 0,42 |
| 24 | — | 8,9 | 0,00333 | 0,37 | |

Eigenverbrauch: 1,2.

*Espe I 128*

Trockengewicht: 127,6 mg frisches Blatt ergaben 60,3 mg trocken.
Einwaage: 111,6 mg.

*Espe I 190*

Trockengewicht: 139,2 mg frisches Blatt ergaben 75,5 mg.
Einwaage: 110,1 mg.

(Tab. 1 Forts.)

*Espe V 34 Blatt-Grösse 5,5 × 4,5 $cm^2$.* — Bestimmungen vom 21.9.50.

Trockengewicht: 144,7 mg frisches Blatt ergaben 65,1 mg trocken.
Einwaage: 118,7 mg.

| Zeit | $a$ | $a - x$ | $k$ | kat.f. = $k/g$ Trockensubst. | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 9,2 | 0,00810 | 1,02 | |
| 16 | — | 8,0 | 0,00789 | 0,98 | 0,98 |
| 24 | — | 7,0 | 0,00767 | 0,96 | |

Eigenverbrauch: 0,8.

*Espe V 27 Blatt-grösse 5,5 × 5,7 $cm^2$*

Trockengewicht: 247,8 mg frisches Blatt ergaben 112,8 mg trocken.
Einwaage: 124,0 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 9,6 | 0,00588 | 0,69 | |
| 16 | — | 8,8 | 0,00530 | 0,62 | 0,65 |
| 24 | — | 7,9 | 0,00548 | 0,64 | |

Eigenverbrauch: 1,2.

*Espe III 29 Blatt-Grösse 6,0 × 6,2 $cm^2$*

Trockengewicht: 175,3 mg frisches Blatt ergaben 70,7 mg trocken.
Enwaage: 122,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 9,3 | 0,00761 | 1,03 | |
| 16 | — | 8,6 | 0,00593 | 0,80 | 0,84 |
| 24 | — | 8,0 | 0,00526 | 0,71 | |

Eigenverbrauch: 1,0.

*Espe III 21 Blatt-Grösse 5,0 × 4,7 $cm^2$*

Trockengewicht: 168,7 mg frisches Blatt ergaben 111,5 mg trocken.
Einwaage: 114,9 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 4,6 | 0,0458 | 4,00 | |
| 16 | — | 2,3 | 0,0417 | 3,65 | 3,83 |
| 24 | — | 1,0 | 0,0437 | 3,83 | |

Eigenverbrauch: 0,9.

*Espe III 58 Blatt-Grösse 4,2 × 3,7 $cm^2$*

Trockengewicht: 120,7 mg frisches Blatt ergaben 62,0 mg trocken.
Einwaage: 105,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 9,8 | 0,00477 | 0,59 | |
| 16 | — | 9,0 | 0,00469 | 0,58 | 0,58 |
| 24 | — | 8,3 | 0,00459 | 0,56 | |

Eigenverbrauch: 1,0.

(Tab. 1 Forts.)

*Espe III 73 Blatt-Grösse 5,7 × 5,5 cm²*

Trockengewicht: 169,4 mg frisches Blatt ergaben 90,0 mg trocken.
Einwaage: 117,8 mg.

| Zeit | *a* | *a* — *x* | *k* | kat.f. = *k*/*g* Trockensubst. | |
|---|---|---|---|---|---|
| 0 | 10,7 | | | | |
| 8 | — | 10,1 | 0,00313 | 0,33 | |
| 16 | — | 9,6 | 0,00294 | 0,31 | 0,32 |
| 24 | — | 9,0 | 0,00313 | 0,33 | |

Eigenverbrauch: 1,3.

*Espe IV 2 Blatt-Grösse 5,5 × 4,7 cm².* — Bestimmungen vom 22.9.50.

Trockengewicht: 125,0 mg frisches Blatt ergaben 52,3 mg trocken.
Einwaage: 96,3 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 8,4 | 0,01464 | 2,44 | |
| 16 | — | 6,5 | 0,01428 | 2,38 | 2,39 |
| 24 | — | 5,0 | 0,01426 | 2,37 | |

Eigenverbrauch: 0,8.

*Espe IV 18 Blatt-Grösse 5,6 × 4,8 cm²*

Trockengewicht: 119,4 mg frisches Blatt ergaben 49,3 mg trocken.
Einwaage: 103,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 3,4 | 0,06374 | 9,1 | |
| 16 | — | 1,5 | 0,05407 | 8,4 | 8,1 |
| 24 | — | 1,0 | 0,04339 | 6,9 | |

Eigenverbrauch: 0,6.

*Espe IV 1 Blatt-Grösse 4,4 × 4,5 cm²*

Trockengewicht: 164,7 mg frisches Blatt ergaben 69,8 mg trocken.
Einwaage: 132,1.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 8,1 | 0,01661 | 1,97 | |
| 16 | — | 6,2 | 0,01556 | 1,85 | 1,85 |
| 24 | — | 4,9 | 0,01463 | 1,74 | |

Eigenverbrauch: 1,2.

*Espe IV 7 Blatt-Grösse 5,5 × 5,0 cm²*

Trockengewicht: 106,6 mg frisches Blatt ergaben 50,6 mg trocken.
Einwaage: 97,8 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 8,1 | 0,01661 | 2,37 | |
| 16 | — | 6,6 | 0,01386 | 1,98 | 2,20 |
| 24 | — | 4,6 | 0,01577 | 2,25 | |

Eigenverbrauch: 1,1.

(Tab. 1 Forts.)

*Espe VIII 3 Blatt-Grösse 5,3 × 4,8 cm²*

Trockengewicht: 106,6 mg frisches Blatt ergaben 61,0 mg trocken.
Einwaage: 95,3 mg.

| Zeit | $a$ | $a - x$ | $k$ | kat.f = $k/g$ Trockensubst. | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 5,7 | 0,03569 | 4,37 | |
| 16 | — | 2,5 | 0,04021 | 4,94 | 4,74 |
| 24 | — | 1,2 | 0,04009 | 4,93 | |

Eigenverbrauch: 0,9.

*Espe VIII 2 Blatt-Grösse 4,5 × 5,9 cm²*

Trockengewicht: 125,8 mg frisches Blatt ergaben 69,0 mg trocken.
Einwaage: 106,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 9,3 | 0,00911 | 1,01 | |
| 16 | — | 6,8 | 0,01305 | 1,45 | 1,31 |
| 24 | — | 5,3 | 0,01321 | 1,46 | |

Eigenverbrauch: 1,1.

*Espe X 8 Blatt-Grösse 4,5 × 4,7 cm².* — Bestimmungen vom 23.9.50.

Trockengewicht: 150,7 mg frisches Blatt ergaben 56,2 mg trocken.
Einwaage: 124,0 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 8,1 | 0,01661 | 2,37 | |
| 16 | — | 5,1 | 0,02086 | 2,96 | 2,84 |
| 24 | — | 3,2 | 0,02234 | 3,19 | |

Eigenverbrauch: 0,9.

*Espe II 111 Blatt-Grösse 6,0 × 6,5 cm²*

Trockengewicht: 183,4 mg frisches Blatt ergaben 75,5 mg trocken.
Einwaage: 111,6 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 9,0 | 0,01089 | 1,55 | |
| 16 | — | 6,8 | 0,01305 | 1,86 | 1,72 |
| 24 | — | 5,6 | 0,01221 | 1,74 | |

Eigenverbrauch: 1,0.

*Espe II 184 Blatt-Grösse 5,7 × 5,8 cm²*

Trockengewicht: 195,5 mg frisches Blatt ergaben 91,7 mg trocken.
Einwaage: 115,3 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 9,4 | 0,00853 | 1,05 | |
| 16 | — | 7,9 | 0,00898 | 1,10 | 1,12 |
| 24 | — | 6,4 | 0,00980 | 1,21 | |

Eigenverbrauch: 0,9.

*Espe IV 10 Blatt-Grösse 5,5 × 5,0 cm²*

Trockengewicht: 155,2 mg frisches Blatt ergaben 75,9 mg trocken.
Einwaage: 116,7 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 11,0 | | | | |
| 8 | — | 9,1 | 0,01029 | 1,20 | |
| 16 | — | 8,1 | 0,008306 | 0,97 | 1,05 |
| 24 | — | 7,2 | 0,00766 | 0,89 | |

Eigenverbrauch: 1,1.

(Tab. 1 Forts.)

*Espe XII 16 Blatt-Grösse 5,5 × 5,0 cm$^2$.* — Bestimmungen vom 25.9.50.

Trockengewicht: 122,9 mg frisches Blatt ergaben 54,3 mg trocken.
Einwaage: 100,1 mg.

| Zeit | $a$ | $a - x$ | $k$ | kat.f = $k/g$ Trockensubst. | |
|---|---|---|---|---|---|
| 0 | 10,0 | | | | |
| 8 | — | 6,2 | 0,02595 | 3,91 | |
| 16 | — | 4,0 | 0,02487 | 3,75 | 3,77 |
| 24 | — | 2,6 | 0,02437 | 3,67 | |

Eigenverbrauch: 0,7.

*Espe XII 14 Blatt-Grösse 5,1 × 5,2 cm$^2$*

Trockengewicht: 134,9 mg frisches Blatt ergaben 54,8 mg trocken.
Einwaage: 101,8 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,0 | | | | |
| 8 | — | 4,8 | 0,02984 | 4,82 | |
| 16 | — | 2,7 | 0,03554 | 5,75 | 5,14 |
| 24 | — | 1,9 | 0,03005 | 4,86 | |

Eigenverbrauch: 0,8.

*Espe X 16 Blatt-Grösse 5,0 × 4,8 cm$^2$*

Trockengewicht: 100,3 mg frisches Blatt ergaben 46,8 mg trocken.
Einwaage: 93,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,0 | | | | |
| 8 | — | 9,3 | 0,00294 | 0,45 | |
| 16 | — | 8,7 | 0,00378 | 0,58 | 0,54 |
| 24 | — | 8,1 | 0,00381 | 0,58 | |

Eigenverbrauch: 0,5.

*Espe XII 9 Blatt-Grösse 4,8 × 3,5 cm$^2$*

Trockengewicht: 126,5 mg frisches Blatt ergaben 60,3 mg trocken.
Einwaage: 114,8 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,0 | | | | |
| 8 | — | 6,6 | 0,02255 | 2,75 | |
| 16 | — | 5,2 | 0,01775 | 2,16 | 2,36 |
| 24 | — | 3,7 | 0,01799 | 2,19 | |

Eigenverbrauch: 0,5.

*Espe XII 2 Blatt-Grösse 4,5 × 4,5 cm$^2$*

Trockengewicht: 137,4 mg frisches Blatt ergaben 68,2 mg trocken.
Einwaage: 99,2 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,0 | | | | |
| 8 | — | 8,9 | 0,00632 | 0,83 | |
| 16 | — | 8,2 | 0,00538 | 0,71 | 0,73 |
| 24 | — | 7,6 | 0,00496 | 0,65 | |

Eigenverbrauch: 0,6

*Espe XII 1 Blatt-Grösse 6,0 × 6,2 cm$^2$*

Trockengewicht: 144,4 mg frisches Blatt ergaben 94,3 mg trocken.
Einwaage: 107,5 mg.

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,0 | | | | |
| 8 | — | 8,8 | 0,00694 | 0,66 | |
| 16 | — | 7,7 | 0,00709 | 0,67 | 0,65 |
| 24 | — | 7,0 | 0,00645 | 0,61 | |

Eigenverbrauch: 0,8.

## *Tab. 2.*

### *Espe I 128 Blatt vom unteren Teil des Baumes.*

| Zeit | *a* | *a* — *x* | *k* | kat.f = *k/g* Trockensubst. | |
|---|---|---|---|---|---|
| 0 | 10,3 | | | | |
| 8 | — | 9,6 | 0,00382 | 0,67 | |
| 16 | — | 8,8 | 0,00427 | 0,75 | 0,74 |
| 24 | — | 8,0 | 0,00457 | 0,80 | |

Eigenverbrauch: 2,5.

Trockengewicht: 157,2 mgr frisches Blatt ergaben 62,4 mgr trocken.
Einwaage: 95,6 mg.

### *Espe I 128 Blatt vom oberen Teil des Baumes (Blatt-Grösse 6 × 6,5 cm²)*

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,3 | | | | |
| 8 | — | 9,1 | 0,00672 | 1,3 | |
| 16 | — | 8,7 | 0,00458 | 0,9 | 1,0 |
| 24 | — | 8,0 | 0,00390 | 0,8 | |

Eigenverbrauch: 1,8.

Trockengewicht: 150,4 mgr frisches Blatt ergaben 54,5 mgr trocken.
Einwaage: 92,3 mg.

### *Espe I 190 Blatt vom unteren Teil des Baumes.*

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,3 | | | | |
| 8 | — | 9,3 | 0,00554 | 0,64 | |
| 16 | — | 8,7 | 0,00458 | 0,53 | 0,56 |
| 24 | — | 8,0 | 0,00457 | 0,53 | |

Eigenverbrauch: 2,2.

Trockengewicht: 140,6 mgr frisches Blatt ergaben 65,5 mgr trocken.
Einwaage: 123,6 mg.

### *Espe I 190 Extrem grosses Blatt (9,5 × 10 cm²) von einem treibenden Ast aus der Spitze.*

| | | | | | |
|---|---|---|---|---|---|
| 0 | 10,3 | | | | |
| 8 | — | 7,7 | 0,01579 | 2,4 | |
| 16 | — | 6,9 | 0,01087 | 1,7 | 2,0 |
| 24 | — | 4,8 | 0,01381 | 2,1 | |

Eigenverbrauch: 3,2.

Trockengewicht: 160,3 mgr frisches Blatt ergaben 58,6 mgr trocken.
Einwaage: 115,4 mg.

Die Blätter der Serie I sind im Durchschnitt grösser als die übrigen untersuchten.

*Tab. 3.*

Zusammenstellung der Katalasebestimmungen.

| Serie u. Nummer | Datum | Blatt-Grösse, $cm^2$ | Aktivität kat.f. | Serie u. Nummer | Datum | Blatt-Grösse, $cm^2$ | Aktivität kat.f. |
|---|---|---|---|---|---|---|---|
| I 128 | 20.9. 50 | 5,8 × 5,3 | 0,95 | V 27 | 21.9. 50 | 5,5 × 5,7 | 0,65 |
| I 190 | 20.9. 50 | 5,3 × 5,5 | 0,42 | V 34 | 21.9. 50 | 5,5 × 4,5 | 0,98 |
| II 111 | 23.9. 50 | 6,0 × 6,5 | 1,72 | V 35 | 20.9. 50 | 4,5 × 5,5 | 1,4 |
| II 184 | 23.9. 50 | 5,7 × 5,8 | 1,12 | V 37 | 20.9. 50 | 5,2 × 4,7 | 1,5 |
| III 21 | 21.9. 50 | 5,0 × 4,7 | 3,83 | VIII 2 | 22.9. 50 | 4,5 × 5,9 | 1,31 |
| III 29 | 21.9. 50 | 6,0 × 6,2 | 0,84 | VIII 3 | 22.9. 50 | 5,3 × 4,8 | 4,74 |
| III 58 | 21.9. 50 | 4,2 × 3,7 | 0,58 | X 8 | 23.9. 50 | 4,5 × 4,7 | 2,84 |
| III 73 | 21.9. 50 | 5,7 × 5,5 | 0,32 | X 16 | 25.9. 59 | 5,0 × 4,8 | 0,54 |
| IV 1 | 22.9. 50 | 4,4 × 4,5 | 1,85 | XII 1 | 25.9. 50 | 6,0 × 6,2 | 0,65 |
| IV 2 | 22.9. 50 | 5,5 × 4,7 | 2,39 | XII 2 | 25.9. 50 | 4,5 × 4,5 | 0,73 |
| IV 7 | 22.9. 50 | 5,5 × 5,0 | 2,20 | XII 9 | 25.9. 50 | 4,8 × 4,5 | 2,36 |
| IV 10 | 23.9. 50 | 5,5 × 5,0 | 1,05 | XII 14 | 25.9. 50 | 5,1 × 5,2 | 5,14 |
| IV 18 | 22.9. 50 | 5,6 × 4,8 | 8,1 | XII 16 | 25.9. 50 | 5,5 × 5,0 | 3,77 |

Die Tabelle 3 zeigt die grossen individuellen Variationen der Espen-Blätter.

## Peroxydase

Die Peroxydase-Aktivität der Espenblätter wurde nach folgendem Verfahren bestimmt:

5 g Blätter wurden fein verreiben und in dest. Wasser suspendiert (ca 50 ml per g Substanz). Von dieser Suspension wurden 5 ml zur Bestimmung verwendet. Zwei 500 ml Erlenmeyer-Kolben wurden mit 0,5 g Pyrogallol (gelöst in 200 ml dest. Wasser) sowie mit 5 ml einer 0,1 %igen $H_2O_2$-Lösung beschickt. In die beiden Kolben, auf der konstanten Temp. = 20° gehalten, werden 5 ml der Blatt-Suspension eingetragen. Die zu messende Reaktion wird durch Susatz von 10 ml 4-norm. Schwefelsäure nach genau 5 bezw. 10 Min abgebrochen.

Die orangerot gefärbte Lösung wird 4—5 mal mit Äther ausgeschüttelt bis die wässrige Schicht vollkommen entfärbt ist. Die Äther-Lösungen werden getrocknet, filtriert und auf 50 oder 100 ml aufgefüllt, je nach der Absorption der Lösung. Die Ablesung geschieht im Photometer mit Grünfilter S 50 gegen eine Standardlösung von Purpurogallin.

*Tab. 4* (Christina Curman). Blätter der Ser. I. No. 190.

| Versuchsdatum | Purpurogallin-Zahl | |
|---|---|---|
| | 5 Min | 10 Min |
| 16. V. 1949 | 0,228 | 0,349 |
| 11. X. 1949 | 0,220 | 0,337 |
| 8. VI. 1950 | 0,251 | 0,344 |
| 28. VI. 1950 | 0,235 | — |

Wie aus den Zahlen der Tab. 4 ersichtlich schwankte die Peroxydase-Wirkung in den Jahren 1949 und 1950 nur unbedeutend.

### Saure Phosphatase

Blätter höherer Pflanzen zeigen im allgemeinen relativ geringe phosphatatische Aktivität (V, GIRI[1]). J. COURTOIS[2], IGNATIEFF u. WASTENAYS[3], PRATESI[4].

Diese Blätter-Phosphatasen werden gewonnen durch Extrahieren oder Auspressen des unter Kühlung gut zerriebenen Materiales. Je 20 der am 10. August 1949 gesammelten Blätter (Grösse 4,5 × 5,0 $cm^2$) der Serien I, II und IV wurden vollkommen gleichmässig mit abgekühltem Toluol- und Äthylacetat-gesättigten Wasser extrahiert und abgepresst. Zur Bestimmung der hydrolytischen Aktivität wurde 0,02 molares $\alpha$-Glycerophosphat als Substrat werwendet und zwar bei 30° unter Einhalten der Acidität $pH = 5{,}5$ und Zusatz von Magnesiumacetat. Kolorimetrische Bestimmung der zu gegebenen Zeiten abgespaltenen Phosphorsäure nach T. TEORELL. In 36 Stunden wurden folgende Mengen abgespaltener $PO_4$ erhalten:

*Tab. 5.* Espen-Blätter.

| | Ser. I | | Ser. II | Ser. IV | |
|---|---|---|---|---|---|
| | 128 | 190 | 111 | 2 | 18 |
| mg $PO_4$ | 4,3 | 4,1 | 4,6 | 3,9 | 3,7 |

Wie schon früher erwähnt wurde, tritt ein wesentlicher Unterschied zwischen den Blättern der diploiden und der triploiden Espen *nicht* hervor.

### Iso-Citrico-Dehydrase und Aconitase (MARTIUS)

Untersucht unter Zusatz von Co-Dehydrase II. Bei Ser. III war die Entfärbungsgeschwindigkeit $100/t$ (reciproker Wert der Entfärbungszeit) der jungen, kleineren Blätter (Mittel 4,2×4,7 $cm^2$) mehr als dreimal so gross als die an den grossen Blättern der Baumspitze gemessene Geschwindigkeit. Bei der Ser. IV trat dagegen ein Unterschied der Dehydrase-Aktivität nicht hervor. Gemessen Mai 1949. Zu weiteren Versuchen reichte unser Vorrat an Co II nicht aus.

## B. Katalase-Aktivität und Entwicklungsgrad von Keimblättern der Gerste nach chemischer Vorbehandlung der Samen bei der Keimung

Versuche über den Einfluss von Phytohormonen, Wuchsstoffen und antibiotischen Stoffen auf die Keimung haben vor einigen Jahren[5] u. a. zur Beobachtung geführt, dass die Keimung in Streptomycin-Lösungen chlorophylldefekte Blätter hervorruft. Dieser Befund, der an mehreren Pflanzen von anderer Seite bestätigt werden konnte[1], wurde dann mit M. BRACCO[2] weiter verfolgt, aber es wurde in unserem Arbeitskreis (ausser Streptidin[3]) noch keine Substanz entdeckt, welcher eine ähnliche Antichlorophyllwirkung zukommt wie dem Streptomycin.

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[1] V. GIRI, Z. physiol. Chem. 245, 185 (1937). — V. GIRI u. N. C. DATTA, Biochem. J. 30, 1089 (1936); Current Science, 4, 833 (1936).

[2] J. COURTOIS et P. DENIS, Enzymologia 5, 288 (1938). — J. COURTOIS et C. PEREZ, Bull. Soc. chim. Biol. 31, 1234 (1939).

[3] IGNATIEFF u. WASTENAYS, Bioc. ek. J. 30, 117 (1936).

[4] P. PRATESI, Ann. di chim. appl. 27, 309 (1937).

[5] EULER, Kem. Arbeten. Ny följd, bd. 9, Mai 1947. — Dieses Arkiv 26 A No. 6 1948.

Da die Untersuchungen dieses Institutes schon vor längerer Zeit ergeben haben, dass die Keimblätter chlorophylldefekter Gersten-Mutanten Katalase-arm sind[4]: haben wir die bezgl. Antichlorophylleffekt geprüften Stoffe auch auf ihre Anti-Katalase-Aktivität hin untersucht; ausserdem haben wir den Einfluss dieser Stoffe auf den Entwicklungsgrad unserer Gerstenpflanzen festgestellt. Hierzu haben wir ausser Siegesgerste noch eine andere reine Gerstensorte, Brage-Gerste, verwendet, welche uns vom Institut für Erblichkeitsforschung, *Svalöv*, freundlichst überlassen wurde.

Wir fügen den in Tab. 6 verzeichneten Ergebnissen noch die Resultate einer kürzlich begonnenen Versuchsreihe an (Tab. 7), die in erster Linie unternommen wurde, um zu untersuchen, ob die chlorophylldefekten Gersten-Mutanten vom Albina Typus eine grössere Empfindlichkeit gegen Streptomycin zeigen als die chlorophyllnormalen Gerstensorten.

**Katalase-Bestimmungen im Keimblatt vom Sieges-Gerste** (Svalöv) K. G. HÖGBERG

3 erste Keimblätter 165 mm lang, ohne Coleoptil wurden in Phosphatpuffer-Lösung verrieben. Eingewogene Substanz: 0,107 g. Trockengew. 7,86 %

| Min | $a - x$ | $k$ | | kat.f = $k/g$ Trockengew. |
|---|---|---|---|---|
| A. Körner, gekeimt in 0,05 % Phloroglucinlösung | | | | |
| 0 | 9,00 | — | | |
| 10 | 7,84 | 0,0060 | 0,0059 | 2,10 |
| 20 | 6,85 | 0,0059 | | |
| 30 | 6,07 | 0,0057 | | |
| 0 | 9,00 | — | | |
| 10 | 7,85 | 0,0059 | 0,0060 | 2,14 |
| 20 | 6,82 | 0,0061 | | |
| 30 | 5,94 | 0,0060 | | |
| B. Parallel-Versuch; Körner gekeimt in Wasser | | | | |
| 0 | 9,00 | — | | |
| 10 | 5,78 | 0,0192 | 0,0188 | 5,41 |
| 20 | 3,79 | 0,0188 | | |
| 30 | 2,51 | 0,0185 | | |
| 0 | 9,00 | — | | |
| 10 | 5,81 | 0,0190 | 0,0186 | 5,35 |
| 20 | 3,82 | 0,0186 | | |
| 30 | 2,58 | 0,0181 | | |

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[1] L. PROVASOLO, S. H. HUTNER u. A. SCHATZ, Proc. Soc. exp. Biol. a. Med. 69, 279 (1948).

[2] M. BRACCO u. EULER, Kem. Arbeten. Ny följd, bd. 10, Okt. 1947 u. Nov. 1947, März 1948.

[3] EULER u. L. HELLER, Dieses Arkiv 1 No. 35 (1949).

[4] EULER u. HARALD NILSSON, Dieses Arkiv 10 B No. 6 1929. — EULER, H. HELLSTRÖM u. D. RUNEHJELM, Z. physiol. Chem. 182 205 1929. — EULER, Hereditas, 13, 61 (1929).

Der Entwicklungsgrad[1], bestimmt am 8. Keimungstag betrug 7 (Wasser-Standard = 10). Wir geben diesen Versuch als Beispiel[2] für die in Tab. 6 u. 7 enthaltenen Zahlen. Im allgemeinen, nicht ausnahmslos, zeigte sich, dass die Katalase-Aktivität der Keimblätter *mit dem durch die gleiche Vorbehandlung erreichten Entwicklungsgrad abnahm.*

*Tab. 6.*

| Gersten-Sorte | Gekeimt in Lösung von | Entwicklungsgrad | kat.f |
|---|---|---|---|
| Sieges-Gerste | Wasser (Kontrollvers.) | 10 | 5,5 |
| | Redukton, 0,1 % | 6 | 4,9 |
| | Askorbinsäure, 0,1 % | 12 | 6,6 |
| Brage-Gerste | Wasser (Kontrollvers.) | 10 | 4,7 |
| | Adrenalin, 0,01 % | 11 | 4,9 |
| | Arterenol, 0,01 % | 10 | 4,5 |
| | Ergothionein (*Stoll*) 0,01 % | 9 | 4,1 |
| | 8-Oxy-Chinolin, 0,005 % | 5 | 3,4 |
| | Chinin 0,01 % | 6 | 4,2 |
| | Phloroglucin, 0,03 % | 4 | 2,8 |
| | Äthylen-glykol | 5 | |

*Tab. 7.*

| Gersten-Sorte | Gekeimt in Lösung von | Entwicklungsgrad | kat.f | Grün : weiss |
|---|---|---|---|---|
| Albina 1 | Wasser (Kontrollvers.) | 10 | 3,0 | 3 |
| | Phloroglucin 0,05 % | 5 | 2,4 | 2,4 |
| *Svalöv* | Orcein | 4 | 1,9 | 2,7 |
| | Streptomycin | 8 | 2,2 | 0,3 |
| | Colchicin 0,05 % | 5 | 1,7 | 3 |
| | Coffein 0,04 % | 4 | 2,8 | 2,9 |
| | Indolessigsäures Na 0,01 % | 8 | — | 3 |
| | Gramin | 7 | 1,7 | 3 |

Das Versuchsmaterial ist noch zu klein als dass man daraus einen bindenden Schluss bezüglich des chemischen Einflusses der untersuchten chemischen Stoffe auf die Chlorophyll-Bildung in den Albina-Mutanten ziehen könnte.

Immerhin scheint sich das Verhältnis Grün : Weiss unter dem Einfluss von Phloroglucin und Crocein ein wenig zu ändern.

Stockholm, Vitamin-Institut und Institut für organ.-chem. Forschung der Universität.

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[1] Vgl. EULER u. ANN.-MARG. PERJE, Dieses Arkiv 20 A, No. 2 (1945).

[2] Die 4 Versuche zeigen die in dieser Serie erreichte Genauigkeit bezw. Reproduzierbarkeit. Bei den bearbeiteten jungen Blättern wird die Inaktivierung durch Verreiben leicht vermieden.

Tryckt den 26 oktober 1951

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# The crystal structure of $K_2SbF_5$ and isomorphous compounds

By ANDERS BYSTRÖM and KARL-AXEL WILHELMI

With 3 figures in the text

The crystal structures of $KSb_4F_{13}$ and isomorphous compounds (1) and of $CsSb_2F_7$ (2) have been reported in two previous papers on complex antimony (III) fluorides.

$K_2SbF_5$ was first prepared by FLÜCKIGER (3). It crystallizes in thin transparent plates and melts without decomposition at 120° C.

$K_2SbF_5$ and the corresponding Rb, Cs, $NH_4$, and Tl compounds also were prepared by dissolving the carbonates (or bicarbonates) and antimony trioxide in the required proportions in dilute hydrofluoric acid and crystallizing at 100° C. Attempts were made to recrystallize $K_2SbF_5$ from a water solution at 18° C, but crystals of $KSbF_4$ were obtained in this way. With a surplus of KF in the solution, crystals of $K_2SbF_5$ were obtained. Single crystals could also be obtained from the melts of $K_2SbF_5$. However, $Tl_2SbF_5$ could also be recrystallized from water solutions without TlF surplus.

The symmetry and cell dimensions were derived from rotation and Weissenberg photographs. The cell dimensions derived from powder photographs (Cr-$K\lambda_\alpha$ = = 2.2909 Å), are:

| | $a$ | $b$ | $c$ |
|---|---|---|---|
| $K_2SbF_5$ | 6.244 ± 0.005 Å | 13.67 ± 0.01 Å | 6.491 ± 0.005 Å |
| $Rb_2SbF_5$ | 6.399 | 13.88 | 6.745 |
| $Cs_2SbF_5$ | 6.652 | 14.45 | 7.099 |
| $(NH_4)_2SbF_5$ | 6.489 | 14.09 | 6.710 |
| $Tl_2SbF_5$ | 6.470 | 14.05 | 6.710 |

Weissenberg photographs of the *hk*0, 0*kl*, 1*kl*, and 2*kl* reflexions indicated that the space group was $D_{2h}^{17}$ — *Cmcm*, provided that the structure is centrosymmetric.

The density of $K_2SbF_5$ was found to be 3.46, compared with the calculated value of 3.54, assuming four formula units in the cell.

The metal atom positions in the Tl compound were first considered. A trial and error process showed, that the Tl and Sb atoms are arranged in the following way:

4 Sb in 4 ($c$): $0y\frac{1}{4}$; $y_1 = -0.086 \pm 0.003$
4 Tl in 4 ($c$): $y_2 = 0.247$ »
4 Tl in 4 ($c$): $y_3 = 0.567$ »

The influence of the fluorine atoms on the intensities is comparatively small in this compound and, as will be seen from Table 1, good agreement is obtained without taking it into account.

The symmetry of $D_{2h}^{17}$ allows for four-fold, eight-fold, and sixteen-fold positions. Twenty F atoms can be situated at:

1) Five four-fold positions
2) Three four-fold + one eight-fold positions
3) One four-fold + two eight-fold positions
4) One four-fold + one sixteen-fold position.

Space considerations for $K_2SbF_5$ — assuming that the distances K–F and F–F cannot be shorter than 2.3 Å — showed that only the last arrangement was in fact possible. Further, to obtain normal F–F distance, the $x$ and $z$ parameters of the sixteen-fold position must must be $\approx \frac{1}{4}$ and $\approx 0$ respectively, and $y \approx \frac{1}{9}$. The calculations indicated, moreover, that the four remaining F atoms are placed in 4 ($c$). The following parameter values for $K_2SbF_5$ could then be derived from the intensities:

4 Sb in 4 ($c$): $0\,y\,\frac{1}{4}$ $y = -0.089 \pm 0.003$
4 $K_1$ in 4 ($c$): $y = 0.250 \pm 0.005$
4 $K_2$ in 4 ($c$): $y = 0.578 \pm 0.005$
4 $F_1$ in 4 ($c$): $y = -0.241 \pm 0.010$
16 $F_2$ in 16 ($h$) $x = 0.222 \pm 0.010$ $y = 0.111 \pm 0.010$ $z = -0.028 \pm 0.010$

*Table 1. $Tl_2SbF_5$*

Comparison between observed and calculated $F$-values. Cu-$K$. The influence of the fluorine atoms is not taken into account

| $h\,k\,l$ | $\lvert F \rvert_{obs}$ | $F_{calc}$ | $h\,k\,l$ | $\lvert F \rvert_{obs}$ | $F_{calc}$ |
|---|---|---|---|---|---|
| 0 2 0 | 50 | —13 | 2.12.0 | 470 | 350 |
| 0 4 0 | 50 | 156 | 2.14.0 | — | 18 |
| 0 6 0 | 640 | —632 | 2.16.0 | 150 | 241 |
| 0 8 0 | 120 | —49 | 3 9 0 | 140 | 219 |
| 0.10.0 | 230 | —233 | 3.11.0 | 100 | 83 |
| 0.12.0 | 430 | 365 | 3.13.0 | — | —4 |
| 0.14.0 | 70 | 19 | 3.15.0 | 320 | —234 |
| 0.16.0 | 170 | 248 | 4.10.0 | 100 | —205 |
| 0.18.0 | 150 | —188 | 4.12.0 | 380 | 324 |
|  |  |  | 4.14.0 | — | 17 |
| 1 9 0 | 400 | 239 | 5 9 0 | 70 | 189 |
| 1.11.0 | 100 | 80 | 5.11.0 | — | 77 |
| 1.13.0 | — | —3 | 5.13.0 | — | —7 |
| 1.15.0 | 360 | —243 | 6.10.0 | 120 | —184 |
| 1.17.0 | 140 | —152 | 6.12.0 | 240 | 296 |
| 2.10.0 | 240 | —232 | 6.14.0 | 120 | 170 |

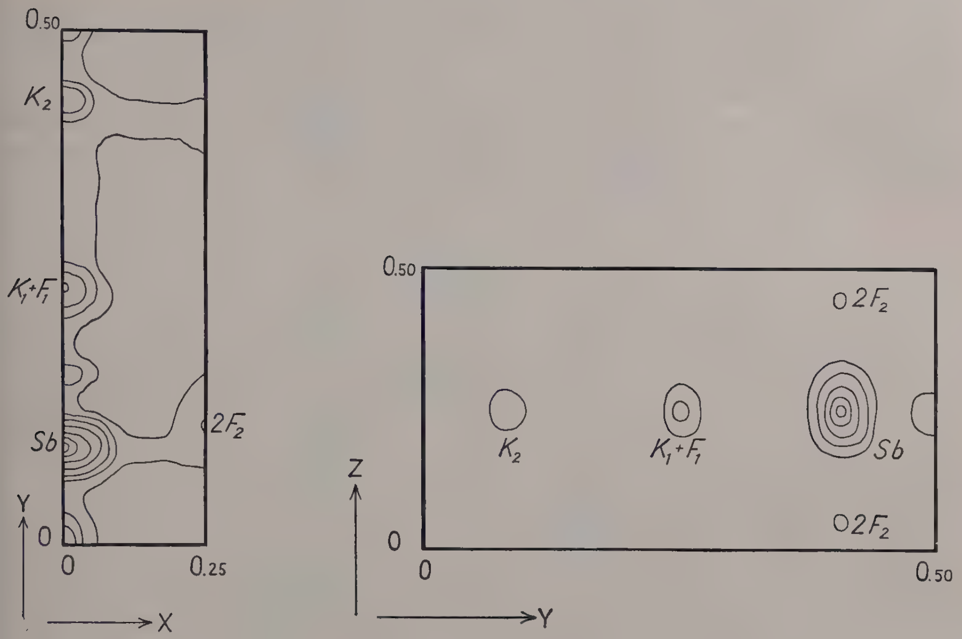

Fig. 1. $K_2SbF_5$. Projection of the electron density on (001) and (100).

As will be seen from Table 2, the agreement between observed and calculated $F$-values is satisfactory.

The electron density projections in (001) and (100) gave within the estimated limits of error the same parameter values for the metal atoms (Fig. 1). The peaks corresponding to the sixteen-fold fluorine atom positions also appear where expected. The fluorine atoms in 4 ($c$) are superimposed on the potassium atoms 4 $K_1$ in both projections. The peaks corresponding to $K_1 + F_1$ atoms are thus higher than the peaks corresponding to the $K_2$ atoms. Two other maxima of the same height as the fluorine maxima appear between the Sb atoms and between the Sb and $K_1$ atoms along the b axis. However, they appear in positions, which cannot, from space considerations, be occupied by fluorine atoms, and may be due partly to errors in the estimated $F$-values because of the absorption and partly to termination of series effects.

The following interatomic distances are obtained:

| | | |
|---|---|---|
| $Sb\text{-}4F_2 = 2.02$ Å ± 0.1 Å | $K_1\text{–}4F_2 = 2.94$ | $K_2\text{–}4F_2 = 2.54$ |
| $Sb\text{-}F_1 = 2.08$ | $K_1\text{–}4F_2 = 2.96$ | $K_2\text{–}4F_2 = 3.42$ |
| | $K_1\text{–}2F_1 = 3.12$ | $K_2\text{–} F_1 = 2.47$ |
| | $K_1\text{–}2F_1 = 3.25$ | |
| Mean value: $Sb\text{-}5F = 2.03$ Å | $K_1\text{–}12F = 3.02$ | $K_2\text{–} 9F = 2.92$ |

The shortest F–F distances are 2.68 Å ($F_1\text{–}F_2$) and 2.77 Å ($F_2\text{–}F_2$).

The structure is shown in Fig. 2. The fluorine atoms around the antimony atoms occupy five of the six corners of an octahedron, so that the antimony atoms lie in bowls of fluorine atoms. The $K_1$ atoms have eight fluorine neighbours at the corners of a prism (distorted cube), and four at the corners of a square. The arrangement of fluorine atoms around the $K_2$ atoms is more distorted, because of the influence of the adjacent antimony atoms, so that rather irregular K–F distances result (see the above table).

*Table 2. $K_2SbF_5$*

Comparison between observed and calculated $F$-values. Cu-$K$.

| $h\,k\,l$ | $\lvert F\rvert_{obs}$ | $F_{calc}$ |
|---|---|---|
| 0 2 0 | 40 | 45 |
| 0 4 0 | 120 | —161 |
| 0 6 0 | 310 | —334 |
| 0 8 0 | 60 | 55 |
| 0.10.0 | 90 | 100 |
| 0.12.0 | 180 | 152 |
| 0.14.0 | — | —50 |
| 0.16.0 | 70 | —41 |
| 1 1 0 | 150 | 126 |
| 1 3 0 | 50 | —41 |
| 1 5 0 | 100 | —123 |
| 1 7 0 | 70 | —65 |
| 1 9 0 | 100 | 72 |
| 1.11.0 | 120 | 91 |
| 1.13.0 | 70 | 28 |
| 1.15.0 | 110 | —75 |
| 1.17.0 | 80 | —68 |
| 2 0 0 | 260 | 206 |
| 2 2 0 | — | —3 |
| 2 4 0 | 30 | 53 |
| 2 6 0 | 140 | —211 |
| 2 8 0 | 40 | —56 |
| 2.10.0 | 50 | 10 |
| 2.12.0 | 190 | 194 |
| 2.14.0 | — | 29 |
| 2.16.0 | 90 | —55 |
| 3 1 0 | 100 | 53 |
| 3 3 0 | — | —1 |
| 3 5 0 | 80 | —59 |
| 3 7 0 | 60 | —68 |
| 3 9 6 | 40 | 25 |
| 3.11.0 | 100 | 75 |
| 3.13.0 | 50 | 52 |
| 3.15.0 | 90 | —59 |
| 4 0 0 | 340 | 282 |
| 4 2 0 | 30 | 32 |
| 4 4 0 | 60 | —85 |
| 4 6 0 | 120 | —224 |
| 4 8 0 | — | 21 |
| 4.10.0 | 40 | 72 |
| 4.12.0 | 110 | 143 |
| 5 1 0 | 100 | 103 |
| 5 3 0 | — | —37 |
| 5 5 0 | 100 | —112 |
| 5 7 0 | — | —43 |
| 5 9 0 | 40 | 77 |
| 5.11.0 | 90 | 77 |
| 6 0 0 | 160 | 161 |
| 6 2 0 | — | 17 |
| 6 4 0 | — | —14 |
| 6 6 0 | 70 | —159 |
| 6 8 0 | — | —21 |
| 6.10.0 | 40 | 28 |
| 7 1 0 | — | 31 |
| 7 3 0 | — | 4 |
| 7 5 0 | 50 | —83 |
| 7 7 0 | 50 | —47 |
| 8 0 0 | 120 | 165 |
| 0 2 1 | 110 | 140 |
| 0 4 7 | 50 | 83 |
| 0 6 1 | 40 | —49 |
| 0 8 1 | 180 | —122 |
| 0.10.1 | 50 | —30 |
| 0.12.1 | 110 | 59 |
| 0.14.1 | 170 | 91 |
| 0.16.1 | — | 0 |
| 0 0 2 | 280 | —204 |
| 0 2 2 | 30 | —23 |
| 0 4 2 | 40 | —65 |
| 0 6 2 | 130 | 214 |
| 0 8 2 | 30 | 56 |
| 0.10.2 | 40 | —9 |
| 0.12.2 | 220 | —180 |
| 0.14.2 | — | —31 |
| 0.16.2 | 80 | —112 |
| 0 2 3 | 50 | —53 |
| 0 4 3 | 40 | —53 |
| 0 6 3 | — | 1 |
| 0 8 3 | 70 | 81 |
| 0.10.3 | 60 | 49 |
| 0.12.3 | 100 | —32 |
| 0.14.3 | 150 | —96 |
| 0 0 4 | 280 | 295 |
| 0 2 4 | 50 | 32 |
| 0 4 4 | 70 | —88 |
| 0 6 4 | 130 | —231 |
| 0 8 4 | — | 24 |
| 0.10.4 | 60 | 72 |
| 0.12.4 | 140 | 142 |
| 0 2 5 | 130 | 130 |
| 0 4 5 | 50 | 76 |
| 0 6 5 | — | —62 |
| 0 8 5 | 60 | —106 |
| 0.10.5 | — | —9 |
| 0.12.5 | 80 | 73 |
| 0.14.5 | 100 | 69 |
| 0 0 6 | 130 | —162 |
| 0 2 6 | — | —19 |
| 0 4 6 | — | 17 |
| 0 6 6 | 100 | 169 |
| 0 8 6 | — | 0 |
| 0.10.6 | 70 | —26 |
| 0.12.6 | 130 | —150 |
| 0 2 7 | — | —27 |
| 0 4 7 | — | —35 |
| 0 6 7 | — | —8 |
| 0 8 7 | 80 | 50 |
| 0 0 8 | 70 | 164 |

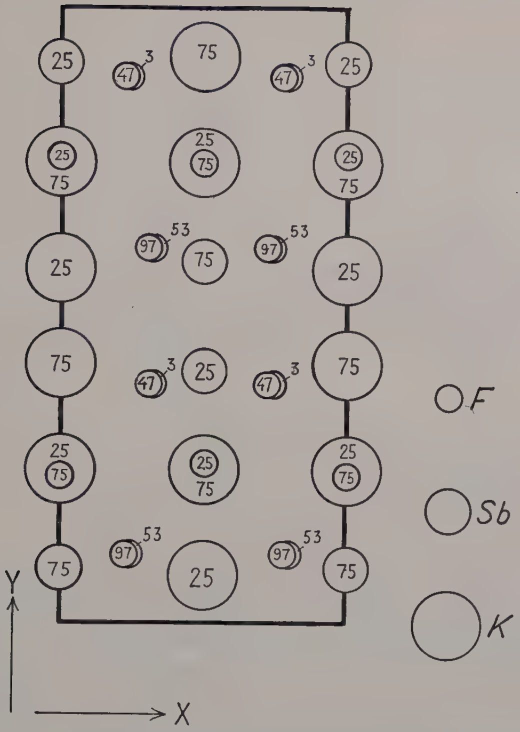

Fig. 2. $K_2SbF_5$. Projection of the structure on (001). Superimposed fluorine atoms are symmetrically displaced. The figures denote the height of the atoms in fractions of c.

The mean distance Sb–5 F = 2.03 ± 0.1 Å is somewhat lower than expected. Only one Sb–F distance is known with accuracy in the hitherto investigated compounds, viz. the distance in the Sb–F–Sb bridge in $CsSb_2F_7$ (2). It amounts to 2.22 ± 0.03 Å and corresponds to a bond-number of 0.5. The Sb–F bonds in $K_2SbF_5$ have a bond-number of 0.6, but one more orbital must be involved in the bond-formation in this case, leading to other types of hybrid-bonds. Thus the distances in the two compounds are not directly comparable. It is of interest to note here that the trivalent Sb-atoms with five covalent bonds in $K_2SbF_5$ have formal charges of –2, the Sb atoms in $CsSb_2F_7$ formal charges of –1, and those in $KSb_4F_{13}$ of 0.

The crystal chemistry of the complex antimony fluorides has some rather unorthodox features. Normally, the central atom is surrounded by a fixed number of ligands situated at the corners of a certain type of polyhedron (tetrahedron, octahedron) and compounds of different formula type are built up of these polyhedra sharing corners, edges or faces. However, the trivalent antimony atom has no fixed coordination number but instead an ability to change its coordination number after the formula type of the compound. Thus we find $SbF_5^{2}$ complex with CN = 5 in $K_2SbF_5$, $Sb_2F_7^-$ groups with CN = 4 consisting of two tetrahedra with one shared corner in $CsSb_2F_7$, and $SbF_3$ groups with CN = 3 loosely joined to $Sb_4F_{13}^-$ complexes in $KSb_4F_{13}$.

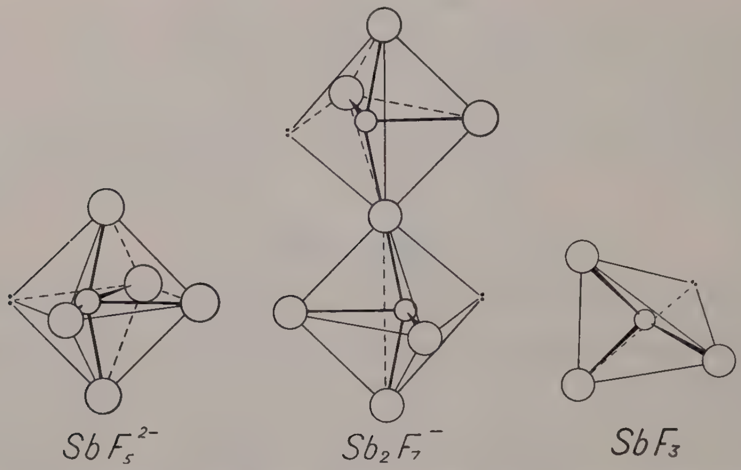

Fig. 3. The configurations of the complex groups $SbF_5^{2-}$, $Sb_2F_7^-$, and of the molecule $SbF_3$.

It seems probable that the types of coordination polyhedra in these compounds is influenced by the presence of a stereochemically active pair of s-electrons. Thus we can describe the configuration of the $SbF_5^{-2}$ complex as an octahedron with the inert pair occupying the sixth corner (Fig. 3 a). Similarly, the $Sb_2F_7^-$ complex may in fact consist of two joined trigonal bipyramides[1] with the inert pairs at two of the four pyramidal apices (Fig. 3 b). The configuration of the independent $SbF_3$ groups, which are found in $SbF_3$ (4) and also loosely joined to a thirteenth F atom in $KSb_4F_{13}$, may thus be described as involving three bonds directed to three of the corners of a tetrahedron with the inert pair at the fourth corner (Fig. 3 c).

The arrangement which was found for the $Sb_2F_7^-$ group in $CsSb_2F_7$ indicates for the compounds $RSbF_4$, $SbF_4^-$ complexes with the fluorine atoms at four of the corners of a trigonal bipyramide. However, another possible arrangement corresponds to that found in tetragonal PbO (Dickinson and Friauf (5), Moore and Pauling (6), Byström (7)) and in $Ag_2PbO_2$ (Byström and Evers (8)): the lead atoms are situated at the apex of a square pyramide with the oxygen atoms at the corners of the base. A third possible configuration for a $SbF_4^-$ group implies five-coordinate Sb atoms (as in the $SbF_5^{2-}$ group) with two of the fluorine atoms shared each between two Sb atoms. The compounds $RSbF_4$ (R = K, Rb, Cs, $NH_4$, Tl) all have different structures and it is thus possible that all these configurations of the $SbF_4^-$ complex may be found to exist. The following data have been determined for the K and Rb compounds:

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[1] With this description of the $Sb_2F_7^-$ complex the Sb atoms might be expected to lie nearer to the equatorial planes of the pyramides than was actually found (2). However the parameters of the F atoms can only be estimated very roughly, especially in the $z$ direction so that it is not possible to determine with any accuracy the location of the Sb atom relative to the equatorial plane.

*Table 3*

| | $a$ | $b$ | $c$ | $\beta$ | Formula units in the cell |
|---|---|---|---|---|---|
| $KSbF_4$ orthorhombic................ | 16.3 | 11.6 | 4.5 | | 8 |
| $RbSbF_4$ monoclinic.................. | 23.3 | 6.2 | 4.6 | 90° | 6 |

As several structure types are also found to exist in the case of the $RSb_2F_7$ compounds, it is obviously of interest to investigate whether some other configuration of the $Sb_2F_7^-$ complex may exist (for instance two joined $SbF_4$-pyramides, corresponding to the alternative arrangement of a $SbF_4^-$ group). $KSb_2F_7$ has a comparatively small unit cell:

*Table 4*

| | $a$ | $b$ | $c$ | $\beta$ | Number of formula units in the cell: |
|---|---|---|---|---|---|
| $KSb_2F_7$ monoclinic.................. | 10.6 | 7.7 | 8.6 | 102° | 4 |

Attempts will be made to solve the structure of these compounds.

This investigation has been supported financially by the Swedish Natural Science Research Council.

## SUMMARY

The crystal structures of $K_2SbF_5$ and the isomorphous compounds $Rb_2SbF_5$, $Cs_2SbF_5$, $(NH_4)_2SbF_5$ and $Tl_2SbF_5$ have been determined.

The influence of the inert s-electron pair on the configurations of the $(SbF_{3+n})^{-n}$ complexes is discussed.

Cell dimensions and symmetry are given for the compounds $KSbF_4$, $RbSbF_4$, and $KSb_2F_7$.

Institute of Inorganic and Physical Chemistry, University of Stockholm, March, 1951.

REFERENCES. (1) **Byström, A.** and **Wilhelmi, K.-A.,** Arkiv Kemi *3,* 17 (1950). — (2) **Byström, A.** and **Wilhelmi, K.-A.,** to be published in Arkiv Kemi. — (3) **Flückiger, F. A.,** Lieb. Ann. *84,* 248 (1871). — (4) **Byström, A.** and **Westgren, A.,** Arkiv Kemi, Mineral. *17 B,* No 2 (1943). — (5) **Dickinson, R. S.** and **Friauf, J. S.,** J. Am. Chem. Soc. *46,* 2457 (1924). — (6) **Moore, W. J.** and **Pauling, L.,** J. Am. Chem. Soc. *63,* 1392 (1941). — (7) **Byström, A.,** Arkiv Kemi, Mineral. *20 A,* No 11 (1945). — (8) **Byström, A.** and **Evers, L.,** Acta Chem. Scand. *4,* 613 (1950).

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# The cytochrome-cytochrome oxidase system of living roots of wheat and corn

By H. Lundegårdh

With 24 figures in the text

## Introduction

This investigation is the continuation of a previous communication on the cytochrome system in living roots (4). A large number of spectrograms have been recorded under variation of the experimental conditions. A selection of these spectrograms is reproduced.

The method of observation was briefly described in the previous communication. The spectrometer was built for automatic recording of the transmitted light intensities at distinct wave-lengths on a Speedomax instrument with the aid of a sensitive photomultiplier tube (EMI 5060). The wave-length screw was automatically moved 5 mμ in intervals of 8 seconds, enabling duplicate readings to be recorded. The reference beam, representing $I_0$, was screened off by means of a diaphragm reducing the intensity to a small fraction. Alternate readings of $I_0$ and $I_1$ were made only for calculation of the "crude spectrum." The light source, a 6 volt lamp burning on a large storage battery, was so constant, however, that good results were obtained from continuous records of $I_0$ in one region of the spectrum, e.g. 390–500 mμ, immediately followed by the continuous recording of $I_1$. This method was regularily adopted for the calculation of the $\frac{\text{ox}}{\text{red}}$-spectrum including two different treatments of the same group of roots.

The wheat seedlings were cultivated in photothermostats and held in distilled water 2–3 days before the experiment. Corn roots were taken from seedlings grown on filter paper in large Petri dishes, moistened with distilled water.

## 1. The $\frac{\text{ox}}{\text{red}}$-spectrum of the cytochrome system

The determination of the absorption spectrum of single components of living cells or tissues is mostly hampered by the simultaneous presence of a number of other coloured substances. A second difficulty is the optical inhomogeneity of most tissues owing to which a direct comparison between the intensity of the transmitted light ($I_1$) and the intensity of the reference beam ($I_0$) may introduce errors caused by multiple reflexion and refraction. As mentioned above the opacity of the living

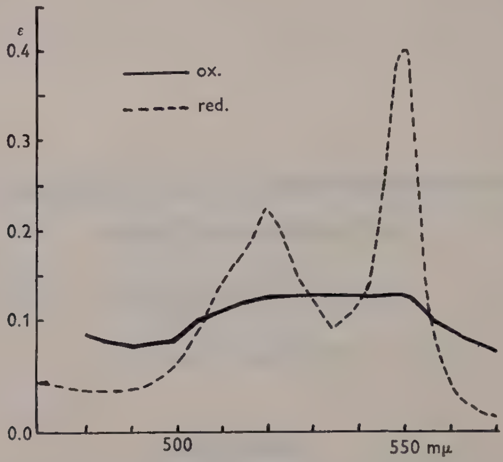

Fig. 1. The α- and β-bands of equimolar quantities of oxidized and reduced cytochrome *c*.

objects necessitates the screening off of the reference beam. The "crude spectrum" of living objects nevertheless creates an important basis for the study of coloured enzymes and our knowledge of some of these, e.g. the cytochrome oxidase, is still almost exclusively limited to this method of observation.

The circumstance, however, that the oxidation-reduction level of the cytochromes is reflected in characteristic changes of the absorption spectrum makes it possible to introduce a method of spectral observation eliminating the interference of all other coloured substances which are not affected by a change in the oxidation-reduction balance.

This method implies the calculation of the $\frac{\text{ox}}{\text{red}}$-spectrum as the difference of the extinctions of the reduced and the oxidized cytochromes present in the object in a quantity which is assumed to be constant during the time of the experiment. It was shown (4 b) that in roots of wheat and corn the cytochromes are partly or even predominantly oxidized, if the objects are held in aerated solutions of neutral salts, e.g. 0.01–0.02 m KCl. Some more cytochrome is turned over in the reduced state if distilled water is used and a complete reduction is obtained in $10^{-5}$ to $10^{-3}$ m. HCN or at complete anaerobiosis. The $\frac{\text{ox}}{\text{red}}$-spectrum calculated from the difference $\varepsilon$ (0.001 m. HCN) minus $\varepsilon$ (0.1 m. KCl) reflects the oxidation-reduction amplitude in the objects and it may be used for the study of the variations in the oxidation-reduction balance induced by salts and various inhibitors. As a fraction of the cytochromes exists in the reduced stage even at maximum intensity of the respiration the $\frac{\text{ox}}{\text{red}}$-spectrum can only with approximation be used for calculations of the absolute quantity cytochrome present in the objects. Only the crude spectrum reveals something of the total cytochrome but it is unfortunately not suited for quantitative purposes. Furthermore the biochemical back-ground of quantitative determinations is still missing except in the case of cytochrome *c*.

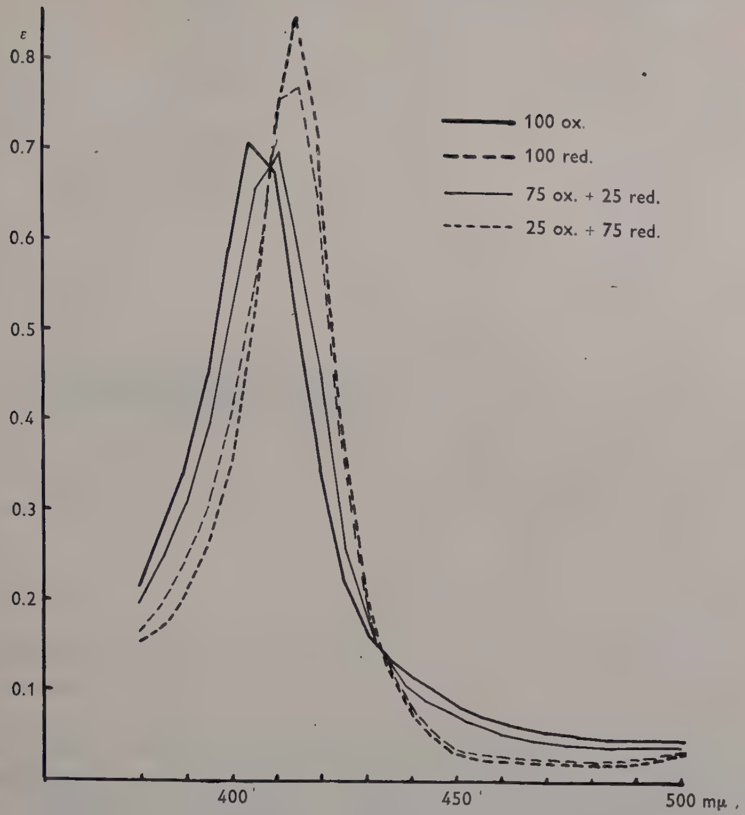

Fig. 2. The γ-band of equimolar mixtures of oxidized and reduced cytochrome *c*, showing the displacement of the band.

Regarding cytochrome *c* the $\frac{\text{ox}}{\text{red}}$-spectrum was determined from a 95 % pure preparation from cow (from the laboratory of Professor Hugo Theorell). The well-known spectrum of this cytochrome shows distinct β- and α-bands in green (c. 520 and c. 550 mμ) only after reduction (fig. 1) whereas the much higher γ-band appears in both stages, but at reduction moved from c. 406 to c. 416 mμ under slight intensification (fig. 2). At partial reduction ( 75 % ox. and 25 % red.) the peak of the γ-band has an intermediate position (fig. 2).

In the calculated $\frac{\text{ox}}{\text{red}}$-spectrum (ε red.–ε ox.) the γ-band appears at 418–420 mμ and its height is only c. 50 % of that of the reduced band at 416 mμ (fig. 3). Of practical importance is the fact that the γ-band of the $\frac{\text{ox}}{\text{red}}$-spectrum remains in its position even at low percentage of reduced cytochrome. It ought to be mentioned that the shape of the $\frac{\text{ox}}{\text{red}}$-curve is to some extent dependent on the dispersion of the instrument in relation to the opening of the slit. The figures refer to a slit of

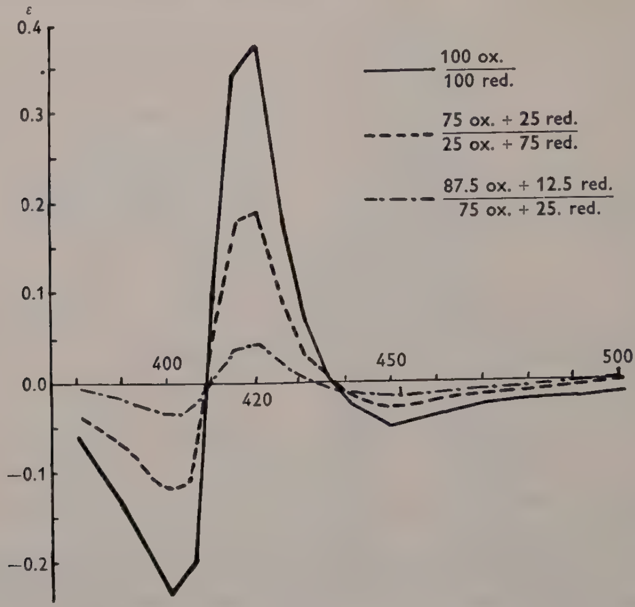

Fig. 3. The $\frac{\text{ox}}{\text{red}}$-spectrum of cytochrome *c*. The minimum at c. 400 and the maximum at c. 420 mμ appear also at partial reduction of the cytochrome. Molar concentration the same as in fig. 2.

0.06–0.10 mm. of a Zeiss monochromator with two quartz prisms and a focal length of 250 mm. In the previous communication (4 b, fig. 4) the slit opening was wider and the peak of the γ-bands is here moved a few mμ to the right.

The extinction at 420 mμ (the recorded interval approaching the peak) of the $\frac{\text{ox}}{\text{red}}$-spectrum is directly proportional to the quantity of reduced cytochrome, provided that the total quantity ox. + red. remains constant, as is normally the case during the time of experiment.

Another characteristic feature of the γ-region of the $\frac{\text{ox}}{\text{red}}$-spectrum is the minimum at c. 400 mμ (fig. 4). The simultaneous appearance of the peak or elevation at 420 and the minimum at 400 together with peaks at c. 520 and c. 550 mμ indicates the presence of cytochrome *c* in living roots of wheat and corn. For identification purposes the magnitude of the relation $\frac{\varepsilon\ 420}{\varepsilon\ 550}$ mμ is useful too. With a slit width of 0.06–0.10 mm it amounts to 3.1 in the pure preparation (in the earlier spectrograms the value was lower; see fig. 4 in 4 b). The root spectrograms on an average showed values of the same magnitude, varying between 2.9–3.2.

Of the other cytochromes only the group $a + a_3$ (3) has been approximately isolated and studied by STRAUB (7). I have tried to calculate the $\frac{\text{ox}}{\text{red}}$-spectrum from STRAUB's curves and the result is shown in fig. 5. The spectrum is of the same type as that of *c* with a miximum at c. 443 mμ and a minimum at c. 410, thus a displace-

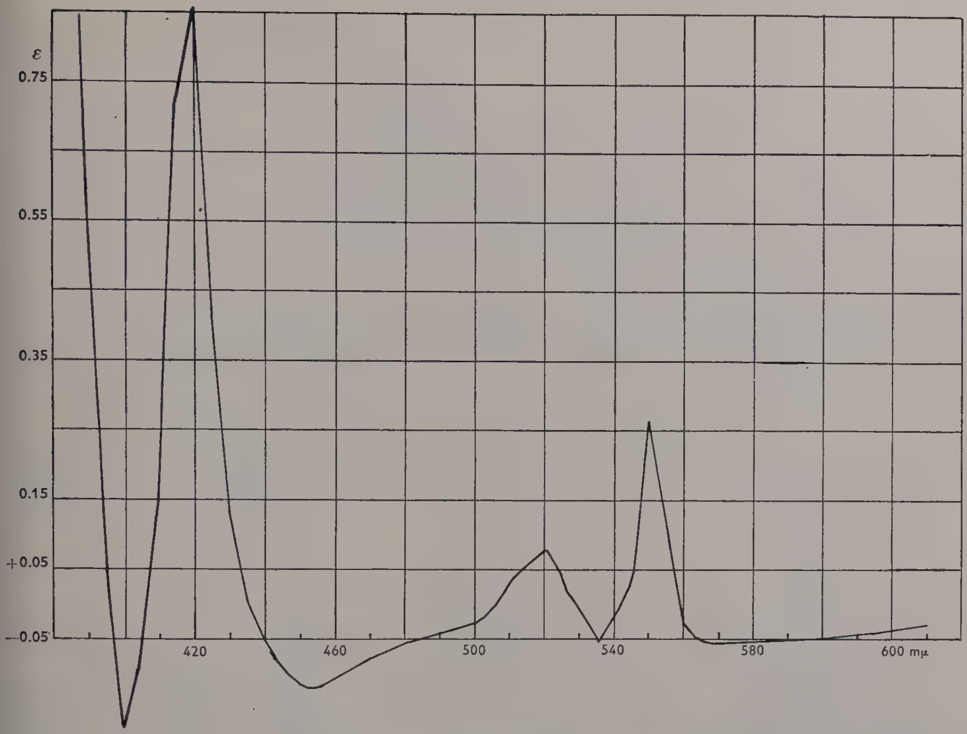

Fig. 4. The $\frac{\text{ox}}{\text{red}}$-spectrum of cytochrome *c*. Isosbestic points appear at 409, 435, 506, 530, 542, and 557 mμ.

ment of 10–15 mμ more than in the $\frac{\text{ox}}{\text{red}}$-spectrum of cytochrome *c*. The broad γ-bands probably indicate the presence of more than one hemin (cf. STRAUB 7) but in spite of the very approximate value of the calculation from STRAUB's small diagrams it shows striking similarities with the corresponding region of the spectrum of the roots. An elevation or a peak invariably appears at 440–450 mμ.

From the $\frac{\text{ox}}{\text{red}}$-spectrograms in figs. 3 and 5 curves can be calculated corresponding to any value of the ordinates 420 and 445 mμ respectively. It is thus possible to calculate the participation of *c* and $a + a_3$ in the $\frac{\text{ox}}{\text{red}}$-spectrograms of the roots (see figs. 6 and 7). What remains of extinction after the subtraction of *c* and $a + a_3$ composes a third γ-band with its peak at c. 430 mμ, approximately corresponding to the γ-band of cytochrome *b*. Unfortunately very little is known about this cytochrome and no dates are available in the literature to be used for the computation of its $\frac{\text{ox}}{\text{red}}$-spectrum. The reports of the position of the bands are varying and modifications of this enzyme may exist. The root spectrum invariably shows a peak at

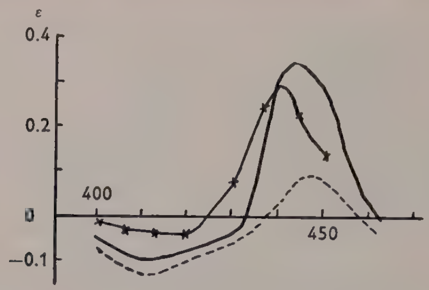

Fig. 5. The $\frac{\text{ox}}{\text{red}}$-spectrum of cytochrome $a + a_3$ calculated from Straub (7). ——— reduced without inhibitor, ——— reduced with CO, - - - - - reduced with HCN.

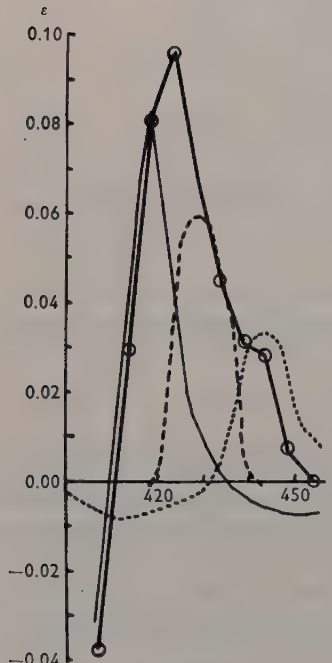

Fig. 6.

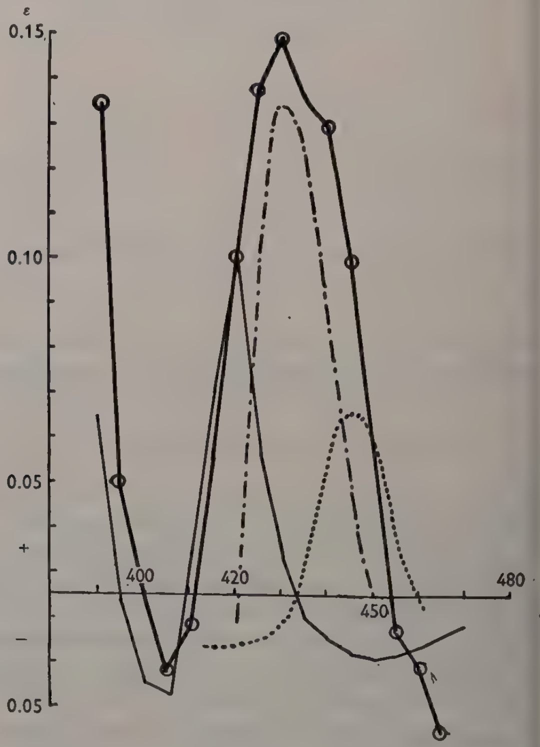

Fig. 7.

Fig. 6. Resolution of the γ-band of the $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots. ——— recorded spectrum $\left(\frac{\text{roots in water}}{\text{roots in HCN}}\right)$, ——— calculated spectrum of cytochrome *c*, - - - - - calculated spectrum of $a + a_3$, — — — — probably the γ-band of a cytochrome *b* appearing after the subtraction of *c* and $a + a_3$ from the observed spectrum.

Fig. 7. Resolution of the γ-band of the $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots. As in fig. 6. The recorded spectrum refers to $\frac{\text{roots in water}}{\text{roots in Na-dithionite}}$.

(565)–570 mμ, approximately corresponding to the α-band of *b*. According to KEILIN and HARTREE it ought to be located at 564 mμ, viz. in my records preferably at 565 instead of 570 mμ.

The difficulty of giving exact figures of the peak of an absorption band was emphasized in the previous communication. The dispersion of the spectroscope in relation to the slit opening are here factors of influence. The modern photoelectric methods yield much better results than earlier direct observations but it is practically impossible to draw a continuous record of sufficient exactitude. Owing to the inertia of the recording instrument a discontinuous record must be chosen and it then depends upon the demands of time how small the wavelength intervals may be. For my purpose the spectrogram had to be recorded as fast as possible, e.g. 100 mμ in little more than 1 minute, and for this reason intervals of 5 mμ were chosen. This means that the measured height of the γ-band of cytochrome *c* at 420 mμ is somewhat lower than the real peak at c. 418 mμ and that the observed peak at 570 mμ, probably belonging to a cytochrome *b*, may as well correspond to a real peak in the region 565–570 mμ. Before the enzyme systems of living tissues are definitely chemically identified the most suitable way of identification seems to be accurate measurements of the extinction at carefully controlled wave-lenghts.

## 2. The crude absorption spectrum

The roots were cut in pieces of 15–20 mm length. 30–100 pieces were pulled together in bundles, just filling the lumen of a glass tube of 4–5 mm diameter. The tube was slightly thinner in the middle part, an arrangement fixing the root bundle firmly enough to resist the slowly flowing stream of the medium through the tube (see 4 b, fig. 3). The spectrum was taken from the central part of the bundle. Consequently no growing ends were exposed. They contain more yellow substances whereas the rest of the root up to a height of several centimeters is fairly uniform in respect of coloured substances including cytochrome, a fact coinciding with the uniform distribution of the anion respiration (4 a).

The crude spectrum (extinction calculated from $I_0$ = "free light" screened off by a diaphragm, $I_1$ = light transmitted through the roots surrounded by an aerated salt solution or distilled water) shows a comparatively small absorption above c. 500 mμ with broad insignificant elevations at c. 530, 550–560, 590–600 and 625–630 mμ (see fig. 8). The two first bands may possibly correspond to bands of reduced cytochrome *b* (530 and 564 mμ according to KEILIN and HARTREE), together with diffuse α- and β-bands of oxidized cytochrome *c* (cf. fig. 1), and the two last bands may belong to $a + a_3$ (cf. WARBURG 9, NEGELEIN & GERISCHER 5, KEILIN 3, YAKUSHIJI & OKUNUKI 10). There is no trace of peroxidases (bands at 548, 583 and 645 according to KEILIN & MANN 2, THEORELL 8) or of other hemins, but the crude spectrum is too indistinct in this region to endure efficient scrutinizing. The β- and α-bands of reduced cytochrome *c* (520, 550 mμ) are absent.

More distinct is the crude spectrum in the region 390–500 mμ. All roots show a distinct band between 470 and 485 mμ with its peak at 480 mμ (± 2.5 mμ). This band is not affected by anaerobiosis or HCN and consequently not related to the cytochrome system or the flavoproteins. From 465 mμ down to c. 390 mμ the light absorption shows a uniform increase interrupted by a distinct band at 440–450 mμ, an elevation or peak at 425 mμ and a similar protuberance at 405 mμ. Of these

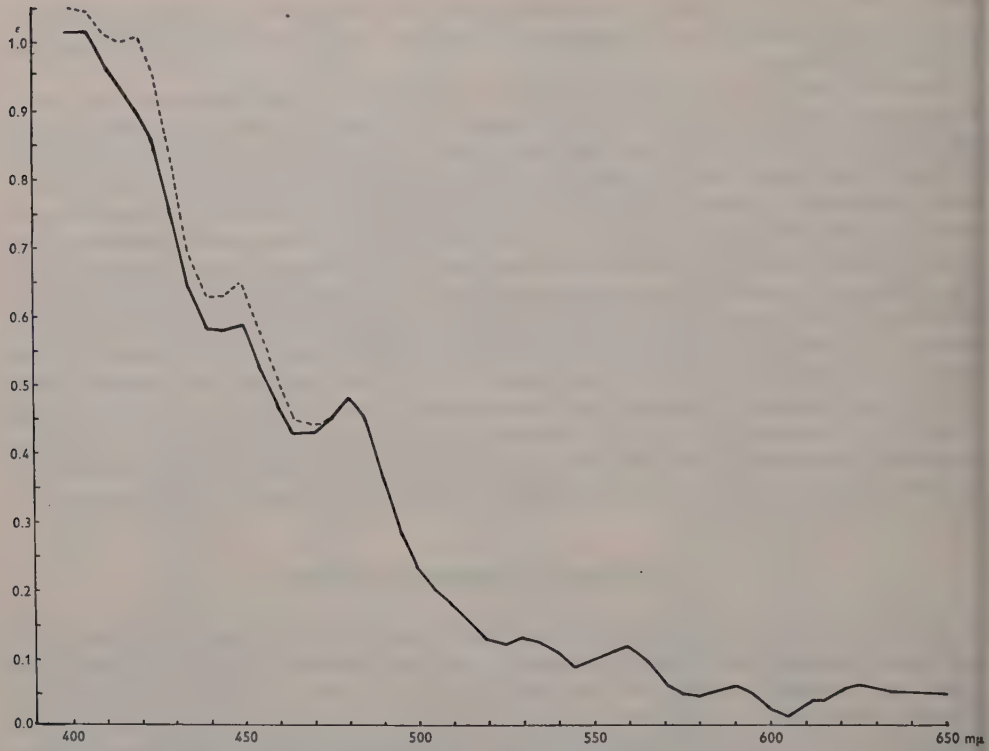

Fig. 8. The crude spectrum of corn roots. ——— roots in distilled water, - - - - - roots in 0.001 m. CO.

bands 440–450 mμ corresponds to the reduced bands of cytochrome $a + a_3$; it grows and moves somewhat to the right under anaerobiosis or in HCN (fig. 9, 10). The band at 425 is also related to the cytochrome system and moves at reduction to 430–435 mμ. It is probably at least in part identical with the γ-band of oxidized cytochrome *b*. KEILIN and HARTREE (3 plate 1, VII) did not recognize the γ-bands of oxidized cytochrome but a γ-band must obviously exist also of *b* and its position ought to be at least 10 mμ to the left of the reduced band. The latter appears in the roots at 430–435 mμ. According to STRAUB (7) and YAKUSHIJI & OKUNUKI (10) the γ-band of oxidized $a + a_3$ is on the other hand located at c. 420 mμ. The elevation at 425 mμ in the crude spectrum is presumably merely a fused band of the oxidized forms of *b* and *a*.

The peak at 405 mμ corresponds to the γ-band of oxidized cytochrome *c* (406 mμ; see above). It disappears after reduction and a strong band appears at 420 mμ simultaneously with the decrease of the elevation at 425 mμ. This behaviour corresponds with the movement of the γ-band at reduction of cytochrome *c* (see above). The broad elevation with its peak at 425 mμ in the reduced crude spectrum corresponds to the fused γ-bands of *c* and *b* (from 418 to 432 mμ).

The crude absorption spectrum thus conveys a schematical picture of the oxidation-reduction balance of the cytochromes. A considerable fraction of $a + a_3$

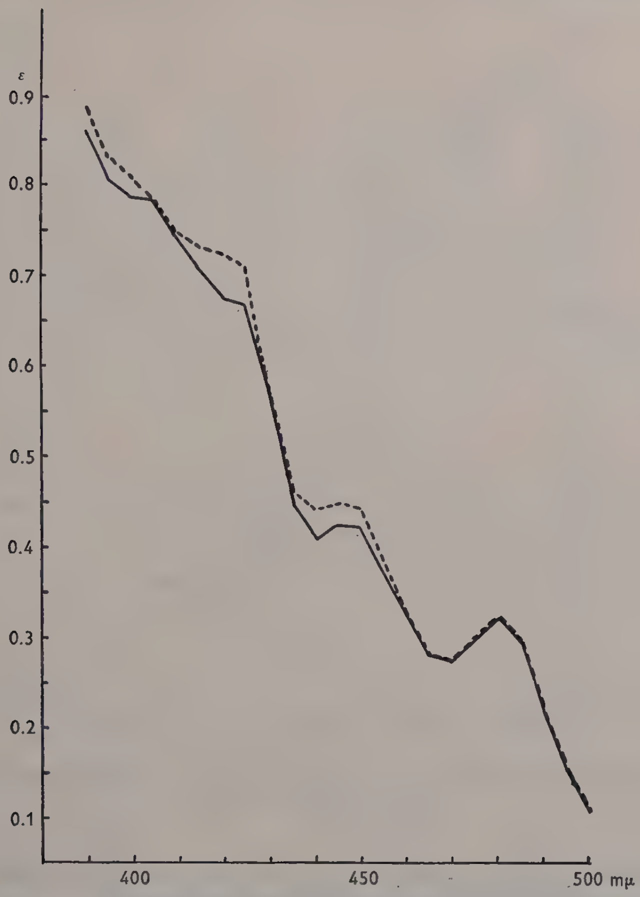

Fig. 9. The crude absorption spectrum of corn roots. ——— roots in distilled water, - - - - roots in 0.001 m. CO.

is probably present in the reduced form, whereas cytochrome *c* seems to be more completely oxidized. Provided our interpretation of the bands is adequate cytochrome *b* is probably more reduced than *c* but to a smaller degree than $a + a_3$. At increasing reduction only the γ-bands of *c* clearly show the expected movement towards higher wave-lengths whereas the corresponding band of $a + a_3$ is enhanced without displacement.

## 3. Further analysis of the $\frac{\text{ox}}{\text{red}}$-spectrum

In more than one hundred experiments series of root spectrograms were recorded, each experiment beginning with aerated distilled water, followed by treatments with solutions of salts or organic acids and finished by complete reduction in cyanide or carbon monoxide. In a number of experiments the effect of anaerobiosis was

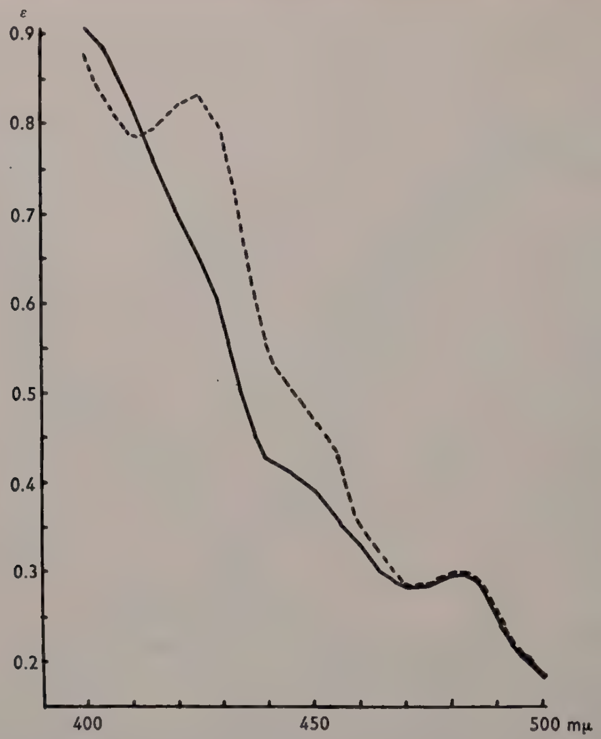

Fig. 10. The crude absorption spectrum of wheat roots. ——— roots in distilled water, - - - - - - roots in 0.001 m. HCN.

studied at complete absence of oxygen. The time course of the inhibition in cyanide and the growing anaerobiosis was recorded in other series.

Positive $\frac{\text{ox}}{\text{red}}$-spectrograms were calculated from $I_0 = \text{ox} =$ the transparency in distilled water, and $I_1 = \text{red} =$ the transp. in HCN etc. All these curves are of the type described under section 1, viz. the bands of the cytochromes are positive indicating a change of the oxidation-reduction-balance in direction of increasing reduction. If the treatments succeeding to the aerated distilled water induce increasing oxidation (this being the case if salts are added) a negative red-spectrum is developed, viz. the bands appear as depressions of the curve instead of elevations.

The diagram in fig. 12 was computed from 25 experiments with HCN, CO or anaerobiosis on the basis of the number of single experiments in which distinct bands or peaks were observed. Four bands dominate, namely 425, 445–450, 550, 555, and 570 mμ. 425 mμ represents the fused $\frac{\text{ox}}{\text{red}}$-bands of cytochromes *c* and *b* (418–420 and 430–435 mμ respectively), 445–450 the fused bands of *a* and $a_3$, 550–555 the α-band of *c* and 570 probably the α-band of *b*. All these bands appear in 50–100 % of the cases. The individual variation is large, however. In several cases the γ-bands of all cytochromes are fused in one broad band with its peak at 425 mμ and small

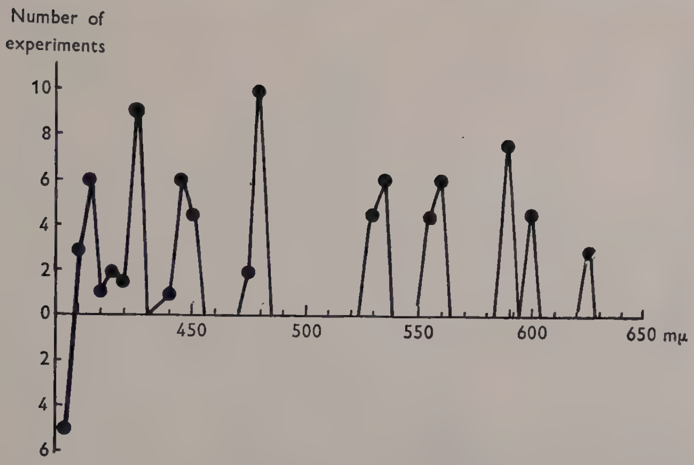

Fig. 11. Frequencies of the more prominent lines in the crude absorption spectrum from 25 experiments.

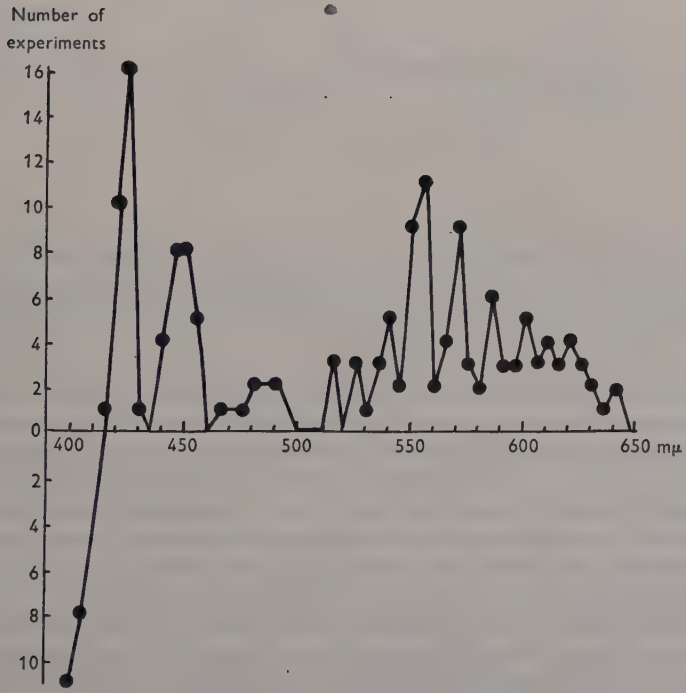

Fig. 12. Frequencies of the lines in the $\frac{\text{ox}}{\text{red}}$-spectrum from 25 experiments.

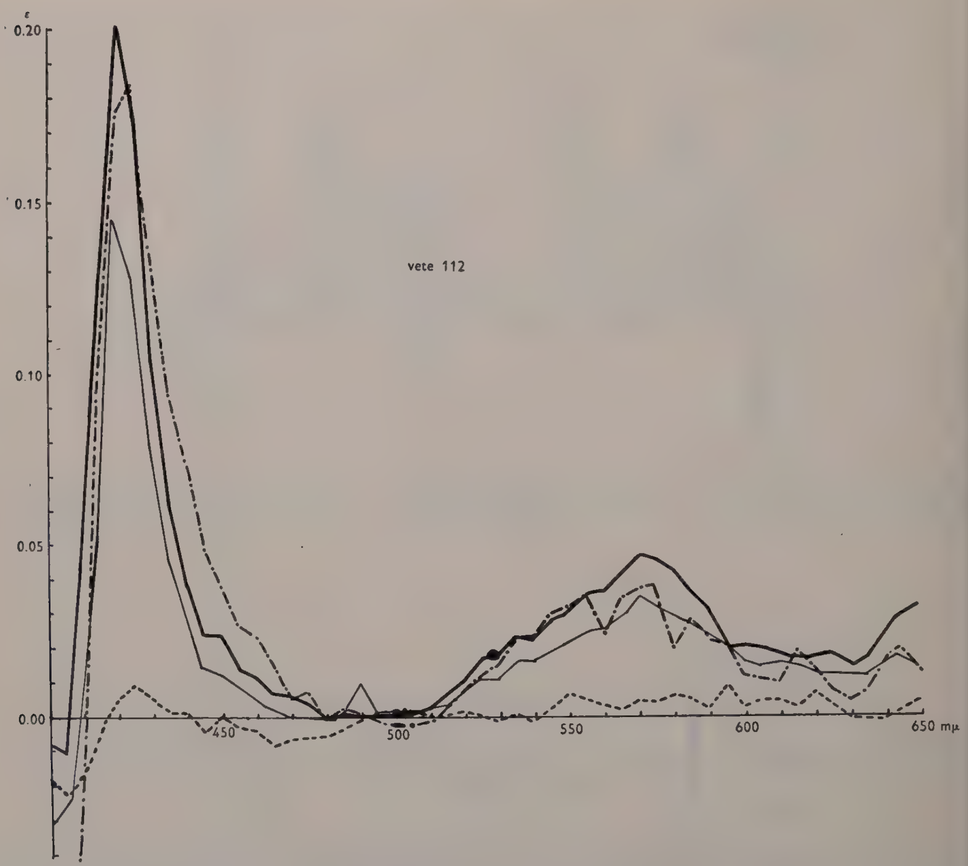

Fig. 13. The $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots at varying supply of oxygen. ox = roots in distilled water, red = roots in $O_2$-free water: - - - - - 15–22 minutes after the begin of the anaerobiosis, ——— 45–52 min. after, ——— 2 hours after, —·—·— transferred to aerated 0.001 m. HCN.

elevations at c. 430 and c. 450 mμ respectively (fig. 13). In some cases the $a + a_3$ band is clearly separated from a much higher fused $c + b$ band. The determination of the time-course of the reduction in HCN or anaerobiosis often reveals the independence of $a + a_3$ (fig. 14, see below). In CO the separation of $a + a_3$ as individual band is more frequently observed (fig. 15). A complete separation of the three peaks at 420, 435, and 450 (corresponding to $c$, $b$ and $a + a_3$) is observed in fig. 16 under the influence of partial anaerobiosis of corn roots.

The general features of the γ-region of the $\frac{\text{ox}}{\text{red}}$-spectrum remain the same irrespective of the method of reduction used. Also sodium hyposulphite lets this curve appear, but the roots of course surrender. A closer inspection, however, reveals special influences of the different inhibitors. As compared with anaerobiosis both HCN and CO (in aerated solutions) convey a considerable increase of the extinction

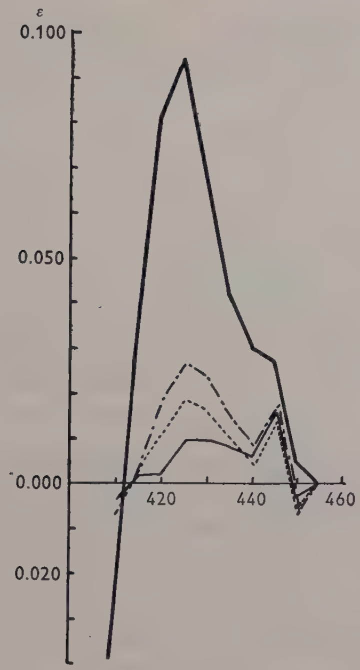

Fig. 14. The time-course of the development of the $\frac{\text{ox}}{\text{red}}$-spectrum in cyanide. From experiments with corn roots. ox = roots in distilled water. red = roots transferred to $10^{-6}$ m. HCN: ——— after 1–50 seconds, - - - - - after 2 minutes, —·—·— after 7 minutes, ——— complete reduction in $10^{-3}$ m. HCN (cf. fig. 17).

in the region of the $a + a_3$ band. A displacement of the band to the left under the influence of CO, as described by STRAUB (7), was not observed. HCN and CO both move the band a few mμ to the right (from 445 to 450 mμ in the records). Another point of disagreement is the fact that neither HCN nor CO are weakening the effect of anaerobiosis. These agents on the contrary increase the extinction. It was on the other hand confirmed that the intensity of the band at 445-450 mμ is higher in CO than in HCN (7). The occasionally observed slight increase of the $b$ γ-band in CO has been interpreted as a special response of the cytochrome oxidase. This effect, however, appears also in HCN. On an average no significant difference could be observed in the response of $b$ to CO or HCN.

Under physiological conditions cytochrome $c$ is obviously not combined with HCN or CO (8, 11). A nearly identical absorption at 415 mμ is attained at all treatments promoting a complete reduction of the system (anaerobiosis, HCN, CO, sodium hyposulphite). For technical reasons the inhibitors were added in aerated solutions. Because of the reversible balance between oxidized and reduced cytochrome no bearing conclusions can be drawn from spectroscopic observations as to the combination of CO with divalent and HCN with trivalent iron. Warburg's conclusion (9, p. 52) that the inhibition with HCN were independent of the oxygen pressure is not valid in the case of roots.

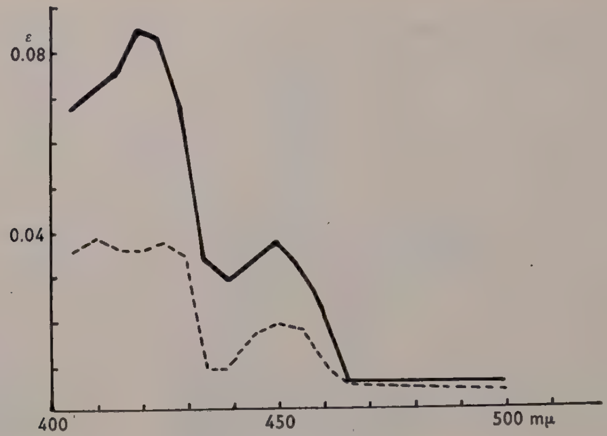

Fig. 15. The $\frac{\text{ox}}{\text{red}}$-spectrum of corn roots. ox = roots in distilled water (aerated), - - - - - red in $10^{-4}$ m. CO, ——— red in $10^{-3}$ m. CO.

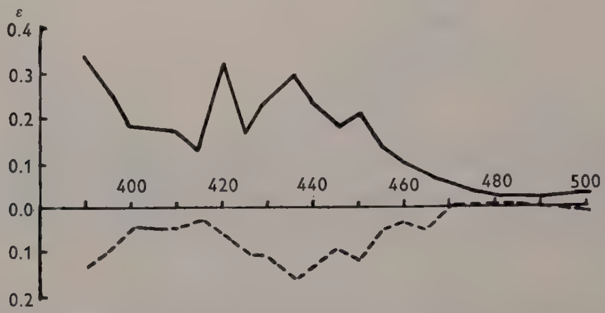

Fig. 16. The $\frac{\text{ox}}{\text{red}}$-spectrum of corn roots. ——— the relation between roots in aerated water and held in $O_2$-free water during 11 minutes, - - - - - the relation between roots held $O_2$-free during 23 minutes and aerated roots.

A remarkable difference between reduction by means of anaerobiosis and reduction by means of HCN or CO is observed in the green part of the spectrum. Whereas both inhibitors produce separate bands at 550 and 570 mμ anaerobiosis develops a single strong band with its peak at 570 mμ (fig. 13). This band is obviously a fusion between a cytochrome band, probably *b* α, and a band of some unknown compound involved in the oxydation-reduction processes but not directly linked to the cytochrome system. This conclusion can be drawn from its time-course. At beginning anaerobiosis (5–11 minutes after shutting off the supply of oxygen) appears a normal cytochrome spectrum with peaks at 550 and 570 mμ (see fig. 13). After more than 11 minutes the unknown compound begins to dominate. After the addition of aerated HCN or CO it disappears again whereas the cytochromes now dominate.

Of the less distinct bands in the region 580–650 mμ one at 585 (± 2.5 mμ) obviously corresponds to the combination between cytochrome oxidase and inhibitor

(CO or HCN; see 11). A weak band is also frequently observed at 600–605 mμ; this is commonly ascribed to cytochrome *a*. It is to be noticed, however, that a band may appear at 585 mμ also under anaerobiosis.

The $\frac{\text{ox}}{\text{red}}$-spectrum of the roots of wheat and corn does not indicate the presence of other cytochromes than the types *c*, *b*, and $a + a_3$, but the indistinct spectrum in green precludes a more detailed analysis. The less significant elevations or bands at 515, 525, and 540 mμ (see fig. 12) may possibly correspond to the β-bands of cytochromes *c* and *b* and a band of cytochrome oxidase at 540 (11). For a complete analysis of the cytochrome system it will be necessary, however, to isolate the components biochemically. Work in this direction is in progress. Also the spectrum in red has to be more closely studied. Unfortunately the sensitivity of the photomultiplier tubes is very low beyond c. 650 mμ.

## 4. The time course of increasing reduction of the cytochrome system

Physiological and biochemical observations indicate a structural co-operation of the cytochromes, these being united to an organized body transmitting electrons from dehydrogenases to molecular oxygen. It is at least very probable that the electrons pass over more than one cytochrome on their way to the oxygen, but the "electron ladder" may have ramifications. If the electron ladder were unbranched the $\frac{\text{ox}}{\text{red}}$-balance of all cytochromes would probably vary synchronously at variations in the oxidation-reduction conditions. Such variations can be brought about by agents controlling the intensity of the dehydrogenases, but the common pathway is control of the oxidation. Even if the observation of the absorption spectrum of living objects creates a guarantee against errors caused by abnormal products of desintegration the possibility is always lurking, however, that other systems than the cytochromes may spectroscopically respond to variations in the oxidation, too. Of good help is here the comparison between $\frac{\text{ox}}{\text{red}}$-spectrograms from anaerobiosis with such from treatments with inhibitors (HCN, CO, Na-hyposulphite), because in the latter case the reduction of the cytochromes, *c*, *b*, and *a* proceeds in the presence of oxygen. In the preceding section an example was given of an absorption band in yellow-green (c. 570 mμ) appearing at anaerobiosis but disappearing in the presence of oxygen, independently of the behaviour of the cytochromes. The reduction of the latter in the presence of inhibitors, on the other hand, implies the development of complex compounds, but here again, as shown above, a comparison with anaerobiosis without inhibitors reveals eventual modifications of the spectrum of the reduced cytochromes and thus opens a further possibility of elucidating fused bands.

The time-course of the reduction was followed by records repeated in increasing intervals, beginning a few seconds or minutes after the entrance of anaerobiosis or the application of solutions of inhibitors. As previously mentioned (4 b) the roots are extremely sensitive to cyanide. The implications brought about at a rise of pH above 7 were previously mentioned, too. The extremely rapid transport of HCN molecules through the tissue means a tremendously enhanced diffusion, probably confined to "adsorption tracks" in the protoplasmic structure. Also oxygen

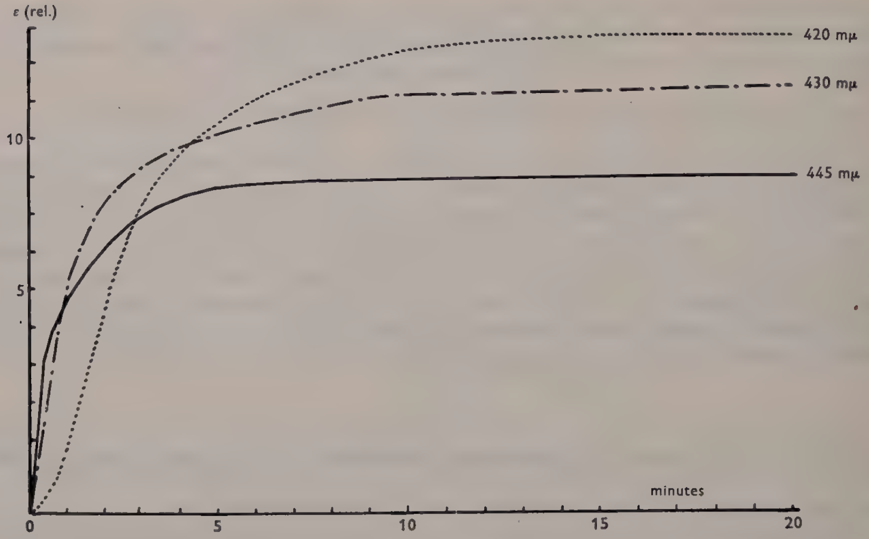

Fig. 17. The course of the development of the γ-bands. From experiments with corn roots and $10^{-6}$ m. HCN (cf. fig. 14).

is rapidly transported, but it is surpassed by HCN. These transport problems of molecules will be more fully treated in a later publication.

The slower transport of oxygen suits our present purpose, but the comparatively slow proceeding of the anaerobiosis (1–2 hours in an $O_2$-free medium before the maximum of reduction is attained) is certainly partly caused by remnants of $O_2$ left in the tissue even after previous evacuation of the intercellular air. The cytochrome oxidase is extremely sensitive to autoxidation and attracts minute traces of oxygen. Possibly also the existence of oxidation-reduction buffer systems besides of the cytochromes, but co-operating with them, contributes to the slow proceeding of the effects of the anaerobiosis.

The experiments on anaerobiosis were carried out according to the following scheme:

1. Determination of $I_0$ = the transparency measured in aerated 0.02 m KCl. 2. Rapid exchange of the salt solution with $O_2$-free distilled water and starting of the time-course records. 3. Sealing of the experimental tube.

In the case of HCN the extremely rapid response impedes the use of complete spectrograms (a full record, from 390 to 650 mμ takes c. 7 minutes.) The important γ-region, from 390 to 460 mμ, is covered in 50 seconds, however, if only single readings are taken in intervals of 5 mμ. In fig. 14 wheat roots were exposed to a streaming aerated solution of $10^{-6}$ m HCN. The first record started a few seconds thereafter, beginning with the highest wave-length. The almost maximal response of the $a + a_3$ band at 445 mμ illustrates the very rapid time-course. The equilibrium of the total cytochrome system is attained after 7–10 minutes. $10^{-6}$ m. HCN inhibits c. 25–30 % of the total activity of the system.

From fig. 14 is clearly seen that the response of $a + a_3$ is considerably more rapid

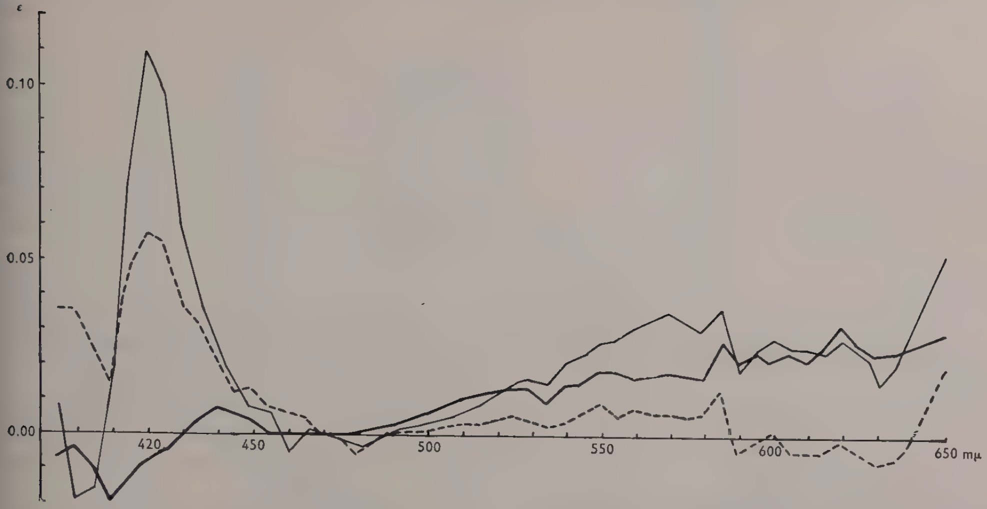

Fig. 18. The $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots. ——— relation between roots in aerated water and $O_2$-free during 5 minutes, ——— $O_2$-free 72 minutes, - - - - - relation between roots in aerated water and treated with 0.001 m. CO.

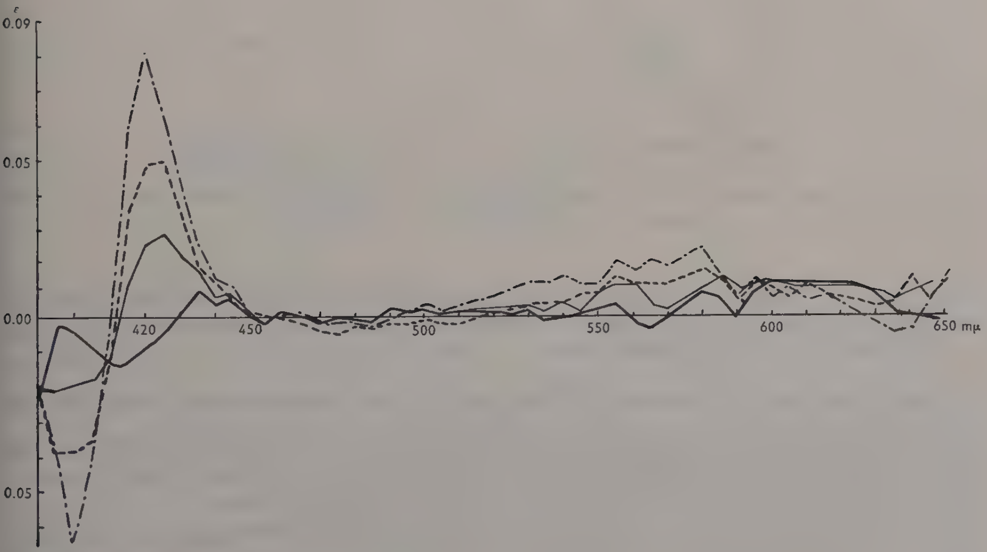

Fig. 19. The $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots, showing the development of anaerobiosis. ——— $O_2$-free 5 minutes, ——— item 30 min., - - - - - item 60 min., —·—·— item 120 min. Early start of $a + a_3$.

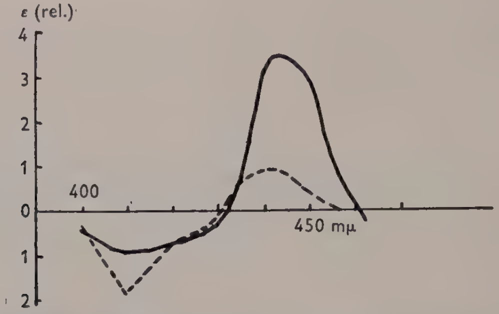

Fig. 20. The observed start period of $a + a^3$ as compared with the results of Straub (7).

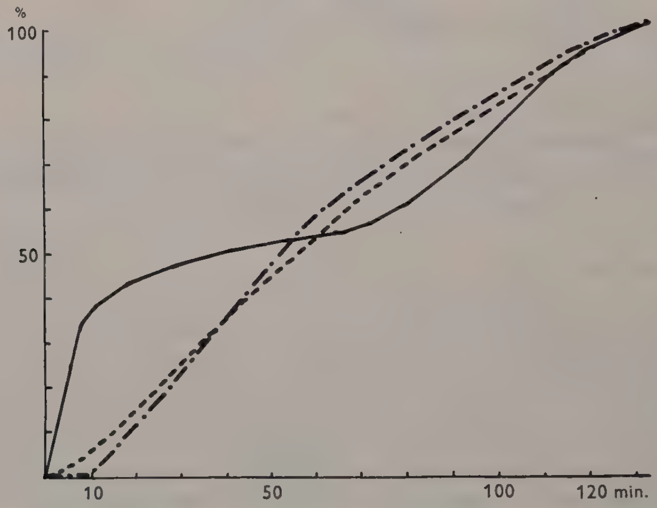

Fig. 21. The time-course of the increasing reduction under anaerobiosis (see fig. 19). ——— 445 mμ, - - - - - 430 mμ, —·—·— 420 mμ.

than that of *b* and *c*. The relative increase of the extinction at 420, 430, and 445 mμ respectively is plotted in fig. 17. The order of time is obviously $a > b > c$.

The time course of the anaerobiosis is illustrated by figs. 13, 18, and 19. In fig. 18 and 19 the early start of $a + a_3$ is reflected in the peculiar form of the $\frac{\text{ox}}{\text{red}}$-curve in the γ-region. It is approximately identical with the corresponding curves calculated according to Straub (fig. 20). A comparison between figs. 17 and 21 shows how similar the time course runs in HCN and under anaerobiosis.

The observations as to the time course of the reduction confirms the results of the analysis of the $\frac{\text{ox}}{\text{red}}$-spectrum based on the experience from animal tissue, yeast, bacteria etc. A new result, however, is the statement of a time order of activation of the different cytochromes, these obviously not being held together in a rigid sys-

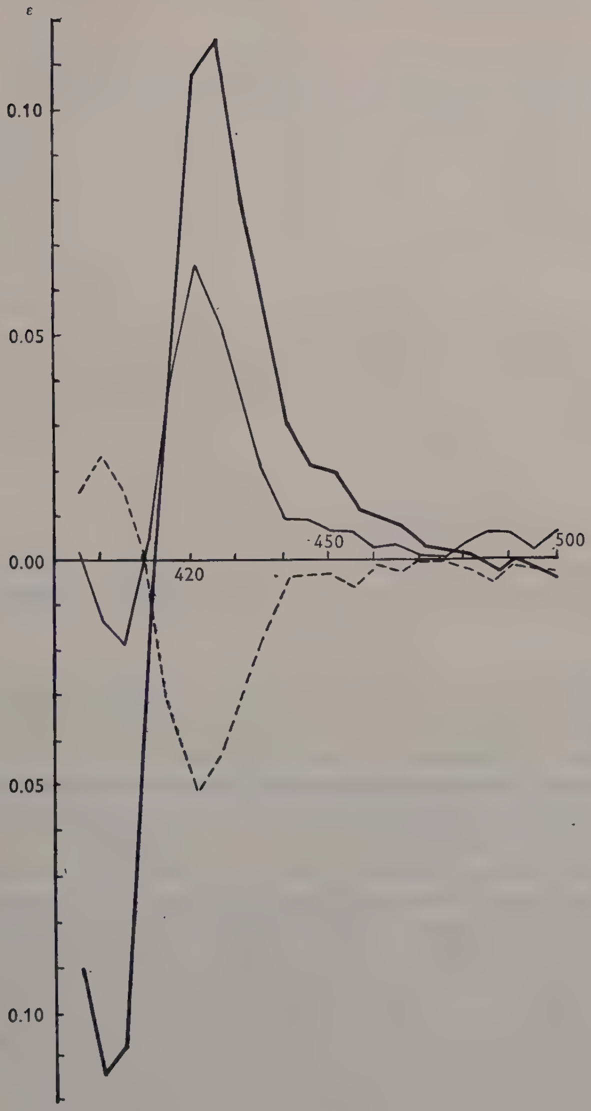

Fig. 22. The $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots ($\gamma$-region). ——— relation between roots in aerated water and $O_2$-free during 24 minutes, - - - - - relation between $O_2$-free during 24 min. and aerated water during 12 min., ——— relation between aerated water and $10^{-3}$ m. HCN.

tem, but revealing the development of characteristic lags of time, between the instantaneously reacting $a + a_3$ on one side and the cytochromes $b$ and $c$, of which apparently $c$ behaves most "independently."

The current idea of the arrangement of the single cytochromes is chiefly based on the biochemical observations of BALL (1) concerning the order of oxidation-reduction

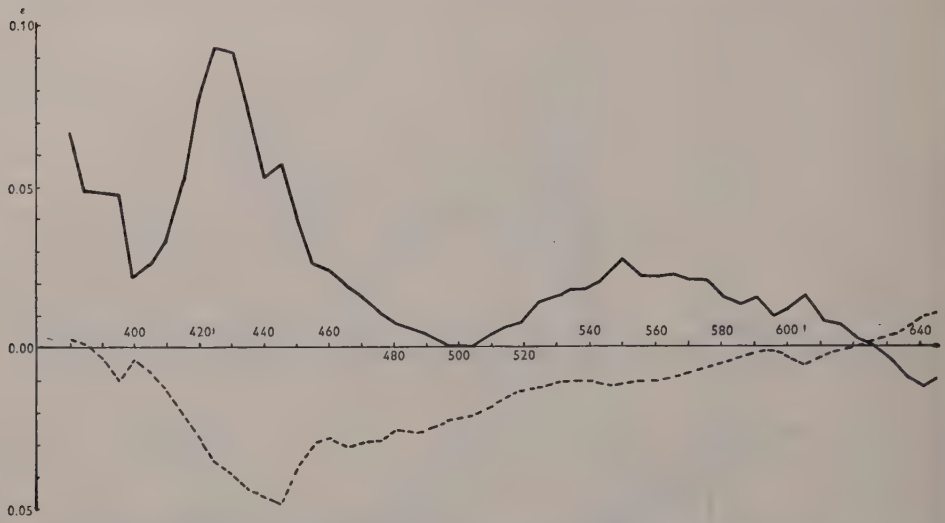

Fig. 23. The $\frac{\text{ox}}{\text{red}}$-spectrum of wheat roots. - - - - - relation between roots in distilled water and in 0.02 m. KCl, ——— relation between 0.02 m. KCl and 0.001 m. HCN.

potentials. Recently SLATER (6 a) has identified cytochrome *b* with the succinic acid dehydrogenase, but I doubt if his conclusions may be transferred to the analogous cytochrome *b* in the roots, because I have not been able to demonstrate any inhibiting influence of malonate or pyrophosphate on the dehydrogenase system co-operating with the cytochrome system (high concentrations were tested at low pH cf. 4 a). Succinic acid indeed stimulates an increasing oxidation quite in analogy with the effects of the anion respiration (see below and fig. 6 in 1).

The results point to the assumption that $a + a_3$ are more closely connected with both the dehydrogenase system and the oxygen, and thus serve as the main path of electron transference from hydrogen to oxygen. Cytochromes *b* and *c* may possibly be linked to side reactions. A ramification of the total enzyme system is probably the explanation of the varying features of the initial phase of rising reduction. Most frequently the event begins with the reduction of $a + a_3$ but cases may also occur in which *c* and *b* are simultaneously affected. The physiological conditions of the experimental material are never quite identical, and variations may be reflected in the mutual oxidation-reduction balance of single cytochromes.

## 5. The response of the cytochrome system to neutral salts

As previously mentioned the search for the real existence of a cytochrome system in roots and other plant tissues was inspired by the investigations on the "anion respiration." The spectroscopical investigations revealed a response of the oxidation-reduction balance of the cytochromes synchronously with the activation of the anion respiration and the active absorption of salts (4 b). The response implied an increase of the balance $\frac{\text{ox}}{\text{red}}$, viz. more cytochrome was transferred to the oxidized

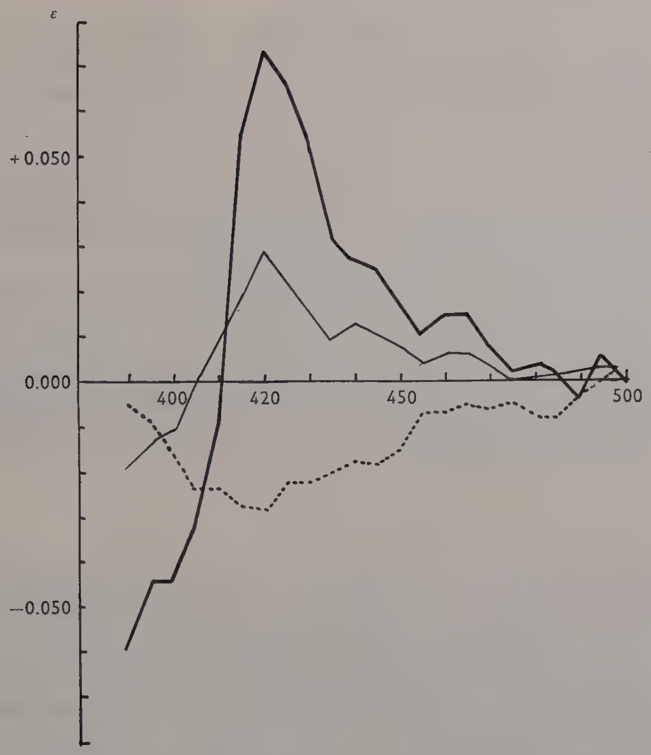

Fig. 24. The $\frac{\text{ox}}{\text{red}}$-spectrum of corn roots. Order of treatments: - - - - - distilled water to 0.02 m. KCl, ——— 0.02 m. KCl to $10^{-6}$ m. HCN. ——— 0.02 m. KCl to $10^{-5}$ m. HCN.

state. The phenomenon is in principle the same as that observed at the return from partial anaerobiosis to aerobiosis. If the spectrum is calculated as log $\frac{\text{roots in anaerobiosis}}{\text{roots in aerobiosis}}$ the curve appears as the reflected image of the $\frac{\text{ox}}{\text{red}}$-spectrum; see fig. 22. The γ-bands of *a*, *b* and *c* are seen as depressions instead of elevations. Similar curves were now obtained from the calculation log $\frac{\text{roots in distilled water}}{\text{roots in 0.01 m. KCl}}$ (see fig. 23, 24). In both cases the "salt curve" is compared with the "cyanide curve" or the anaerobiosis curve, viz. the completely or partially reduced system. All curves refer to zero (= the abscissa) = the extinction in distilled water.

The figures 23 and 24 clearly show a deep depression at 445–450 mμ and a comparatively modest depression at 420–430 mμ. Postulating the identity of the bands with corresponding types of cytochromes it must be concluded that the presence of salts conveys a comparatively strong oxidation of $a + a_3$. The response of the band at 605 mμ is shown in fig. 23.

A computation of thirteen preliminary experiments resulted in the following average values of the height of the γ-bands at wave-lengths corresponding to the peaks of *c*, *b* and $a + a_3$.

| λ | 420 | 430 | 435 | 440 | 445 | 450 mμ |
|---|---|---|---|---|---|---|
| Ext. salt — ext. dist. water (× 10) | −0.114 | −0.132 | −0.125 | −0.127 | −0.136 | −0.121 |
| Ext. HCN — ext. dist. water (× 10) | +0.711 | +0.768 | +0.622 | +0.492 | +0.365 | +0.305 |
| Depression in salt in % of elevation in HCN | 16 | 17 | 18.5 | 26 | 37 | 40 |

The response of $a + a_3$ is 2–3 times as high as the response of $c$ and $b$. Owing to a less effective pretreatment of these roots the anion respiration (and salt absorption) was low. Later experiments rendered higher values.

In fig. 24 the depression of the reduction of the cytochromes developed in salt solution was compared with the positive effect of HCN. The "salt curve" is on an average the negative copy of the curve obtained with $10^{-6}$ m HCN, a concentration here producing c. 50 % reduction of the cytochrome system. But also in this case the form of the curve is changed, showing a more intensive response of $a + a_3$. The relative figures of the oxidation in salt solution as compared with the complete reduction (in $10^{-5}$ m HCN) are:

| λ | 420 | 430 | 445 mμ |
|---|---|---|---|
| | 40 % | 49 % | 73 % |

The unanimous results of the experiments with neutral salts and salts of organic acids (see 4 b, fig. 6) support the idea of the response of the oxidation-reduction balance of the cytochrome system being identical with the "anion" or "salt respiration." The order of the response is $a + a_3 > b > c$, similar to the order of the response to increased autoxidation of the cytochrome oxidase dealt with in the previous section, but with still more pronounced acceleration of the system $a + a_3$.

As the absolute quantities of the cytochromes present in the measured transverse section of the root bundle is not known no accurate calculation is yet possible of the displacement of the oxidation-reduction balance under the influence of the salts, but an approximate estimation may be based on the height of the bands of $a + a_3$ in the crude spectrum (see above).

In ten experimental series with corn roots the approximate height at 445 mμ was on an average 0.061 (in positive extinction values). In the $\frac{\text{ox}}{\text{red}}$-spectrum $\left(\frac{\text{aqua}}{\text{HCN}}\right)$ the height amounted to 0.123. As oxidized $a + a_3$ has a very low light absorption at 445 mμ these figures mean that the total quantity of $a + a_3$ corresponds to the extinction value 0.061 + 0.123 = 0.184. The oxidation reduction balance in roots held in distilled water is then $\frac{\text{ox}}{\text{red}} = \frac{123}{61} = \text{c. } 2 =$ about 67 % oxidized.

The presence of salts in the medium now promotes a further oxidation lowering the extinction to only 0.034 or c. 50 % of the value in distilled water. At full intensity of the anion respiration thus only 0.061–0.034 = 0.027 or c. 15 % of the total quantity of $a + a_3$ remains reduced.

Owing to the "distilled water respiration" (see 4 a), viz. an idling maintained by anions circulating in the roots, the actual fluctuations due to the presence or the absence of movable anions are about four times as large as those induced by salts absorbed from the medium. It is consequently to be expected that at complete absence of movable anions (= zero level of the anion respiration) the cytochromes will predominantly turn over to the reduced state. Expressed in electro-chemical terms the variations in oxidation-reduction potential caused by the presence or the absence of salts in the medium amount to 10–15 mv. In objects where the idling is low the potential amplitude may be considerably higher. It is under these circumstances not surprising that a number of processes are correlated with the active transport of salts through the tissues (cf. 4 a).

In the experiment plotted in fig. 24 the crude spectrum showed a height of the reduced band of $a + a_3$ amounting to 0.040. In HCN a further increase of 0.026 was added, or totally 0.066. The anion respiration in 0.02 m. KCl lowered the value to 0.021, resulting in a balance $\frac{\text{ox}}{\text{red}} = \frac{66-21}{21} = 2.1$. In the absence of salts in the medium the balance sank to $\frac{66-40}{40} = 0.65$. If also the "native anions" (cf. 4 a) could be eliminated probably values lower than 0.1 would result, implying an almost complete reduction of the cytochrome system. The change in oxidation-reduction potential at a shift in the balance from 2.1 to 0.65 is c. 30 mv.

## 6. Conclusions

The results leave no doubt as to the existence in the roots of an oxidation-reduction system carrying all the essential qualities of a complete cytochrome-cytochrome oxidase system. It is furthermore quite obvious that this system is composed by three main components behaving very similarily to the well-known cytochromes $a + a_3$, $b$ and $c$ even if a definite biochemical identification cannot be made before the enzymes are isolated and physico-chemically characterized. Our present task is not so far reaching but primarily limited to the demonstration of the participation of the system in the anion respiration, and in this connexion to a study of the activity of the components.

According to the theory of anion respiration the cytochrome system, or at least a part of it, is organized as an electron ladder. The electrons ought to move through the body of the organized system very much similar to the movement through a metallic conductor. The movement of free anions, surrounding the system, in the opposite direction to that of the electrons is an electro-chemical postulate (cf. 4 b): the activity of movable anions regulates the "density" of the electron stream and the density of the electron stream concomittantly regulates the intensity of the anion respiration and the active transport of salts.

We know very little about the kinetics of the cytochrome system. Biochemically even the normal $\frac{\text{ox}}{\text{red}}$-balance was not yet studied. The following consideration may serve as a basis for attacks upon this problem.

The cytochromes have an intermediate position between a pole of reduction, the dehydrogenases, and a pole of oxidation, the oxygen. The $\frac{\text{ox}}{\text{red}}$-balance of each cytochrome, viz. the molar relation between the oxidized and reduced fractions, is of course outlined by the specific oxidation-reduction potential but in the operating system the balance is raised or lowered in relation to the power of reduction and the power of oxidation. If only the velocity of electron transference through the complete system varies the $\frac{\text{ox}}{\text{red}}$-balance of each cytochrome remains unchanged even if the intensity of respiration varies. This is an important conclusion because it means that variations in the intensity of the aerobic respiration are not necessarily reflected in the spectroscopical behaviour of the cytochromes.

Variations in the intensity of the aerobic respiration will be reflected in changes of the $\frac{\text{ox}}{\text{red}}$-balance of cytochromes only if the electron stream is changed anisotropically viz. if the influx of electrons from the dehydrogenases is accelerated or retarded in comparison to the outflow of electrons to the oxygen. Experiments in vitro have shown that inhibitors of the dehydrogenase system, e.g. malonate or pyrophosphate in the case of succinic acid dehydrogenase, change the balance in the direction of an increasing oxydation of the cytochromes, if the cytochrome oxidase remains unaffected. Inhibition of the cytochrome oxidase, on the other hand, shifts the balance to the side of reduction, if the dehydrogenase system still maintains its reducing qualities.

If now the presence of salts uniformly accelerated the transference of electrons through the whole system variations of the salt concentration would not be reflected in variations of the oxidation-reduction balance of the cytochromes. If preferably the dehydrogenases were stimulated the relation $\frac{\text{ox}}{\text{red}}$ would sink, viz. the reduction level would rise. The experiments show the reverse. As the effect of salts is primarily an increase of the transference of electrons, reflected in an increased respiration, the explanation cannot be a retardation of the influence of the dehydrogenases, however, but an increase of the outflow of electrons from the cytochrome oxidase to oxygen.

More difficult is to decide whether the potential gap between cytochrome oxidase and oxygen or the gap between cytochrome oxidase and cytochrome *a* is the main "sleuce of electrons." The outflux of electrons from cytochrome oxidase to oxygen is compensated by the immediately following junction between the activated oxygen and protons, the synchronical supply of which can be traced back to the bottom of the potential ladder, viz. the oxidation of the hydrogen by the first cytochrome of the chain (see the scheme in 4 b p. 70). But in the moment the $\text{Fe}^{++}$ of the cytochrome oxidase looses one electron the increase of one positive charge of the Fe- atom must be balanced by an anion and the one nearest at hand is a free moving anion from the surroundings of the enzyme system. In other words: the oxidized enzyme functions as a carrier of anions. The next step back in the potential ladder is the reduction of the cytochrome oxidase by reduced cytochrome *a*. At the exchange of one electron between the $\text{Fe}^{+++}$ of the oxidase and the $\text{Fe}^{++}$ of the cytochrome the carried anion is then moved one step further. This process is repeated in the subsequent steps, the last cytochrome of the system finally being reduced by one hydrogen atom from the dehydrogenase system.

This intermittent attraction and repulsion of movable anions from the side of the operating cytochromes must not necessarily imply the forwarding of the anions in a certain direction, however, because these can also be figured circulating between the steps of the ladder. An active transport will be realized only if the transference of the anion from one enzyme to the next simultaneously implies the crossing of a barrier preventing the anion to move back to its former position. Such a barrier can have the character of a monomolecular layer, perhaps formed by the cytochrome oxidase.

The observed phenomena support the idea of the steps of the potential ladder most closely related to the oxygen being most intimately dependent on the presence of movable anions. Cytochromes $a + a_3$ are obviously not able to counterbalance the electronic pulsations in any other way than by intermittent attraction and repulsion of freely movable anions. If the oxidase is organized in a layer of oriented molecules coating a surface of the complete enzyme system anions are picked up from the medium synchronously with the activation (ionization) of the oxygen molecules. In the phase of oxidation of the cytochrome oxidase the anions are thus moved from the medium across the barrier of surface forces formed by the organized complex. There is ample evidence of the existence of a stoechiometrical reciprocity between the number of anions forwarded across the barrier and the numbers of activated (or consumed) oxygen molecules (4 a). The energy needed for the crossing of the barrier represents probably only a small fraction of the total energy developed at the junction of the activated oxygen and the protons, even if this means a considerable rise of activity (concentration).

The spectroscopic observations are still too incomplete to allow a distinction between the time reactions of cytochrome oxidase and cytochrome *a* and the question must accordingly be left open if the key rection of the anion respiration is the oxidation of cytochrome oxidase by oxygen or if also the further step from cytochrome oxidase to cytochrome *a* is involved in the stoechiometrical exchange between electrons and anions.

As to the cytochromes *b* and *c* the less prompt response upon variations in the oxidation is most probably caused by reversible side-reactions, e.g. oxydative phosphorylation (cf. 6 b), creating a sort of buffer mechanism maintaining a less sensitive $\frac{\text{ox}}{\text{red}}$-balance of these enzymes. If these side-reactions are preferably linked to cytochrome *c*, and *b* is placed between *c* and the dehydrogenases this would mean a partial screening off also of *b* from sudden variations in the $\frac{\text{ox}}{\text{red}}$-balance of the $a + a_3$ system.

## SUMMARY

A useful tool in identifying the presence of the cytochrome system in living objects and its response to changes in the oxidation-reduction conditions is the $\frac{\text{ox}}{\text{red}}$-spectrum, calculated as the difference in extinction between the reduced and oxidized stages. The $\frac{\text{ox}}{\text{red}}$-spectrum of biochemical preparations of the cytochromes *c* and $a + a_3$ was calculated and compared with corresponding records from living roots of wheat and corn. As far as the spectroscopical analysis can be trusted the roots are provided with a complete cytochrome system with components $a + a_3$, *b* and *c* corresponding to the types distinguished in objects of animal origin or micro-organisms.

Recording of the time course of increasing reduction or oxidation reveals a difference between the group $a + a_3$ on one side and the cytochromes *b* and *c*, the former immediately responding to changes in the conditions, the latter often slower.

The presence or absence of movable anions (salts, organic acids) in the medium regulates the oxidation-reduction balance of the cytochrome system, the oxidation being promoted in the former case. The relation of this phenomenon to the "anion respiration" is discussed.

REFERENCES. 1. **Ball, E. G.,** Biochem. Zeitschr., *295*, 1939, 262. — 2. **Keilin, D. & Mann, T.,** Proc. Roy. Soc., B, *122*, 1937, 119. — 3. **Keilin, D. & Hartree, E. F.,** Proc. Roy. Soc., B, *127*, 1939, 167. — 4 a. **Lundegårdh, H.,** Ann. Roy. Agr. Coll. Sweden, *16*, 1949, 372. — 4 b. —— Ark. f. Kemi, Kungl. Svenska Vetenskapsakad., *3*, 1951, 69. — 5. **Negelein, E. & Gerischer, W.,** Biochem. Zeitschr., *268*, 1934, 1. — 6 a. **Slater, E. C.,** Biochem. Journ., *46*, 1950, 484. — b. —— Nature, *166*, 1950, 980. — 7. **Straub, F. B.,** Hoppe-Seylers Zeitschr. f. physiol. Chemie *268*, 1941, 227. — 8. **Theorell, H.,** Ergebn. d. Enzymforsch., *9*, 1943, 231. — 9. **Warburg, O.,** Schwermetalle als Wirkungsgruppen von Fermenten. Berlin 1946. — 10. **Yakushiji, E. & Okunuki, K.,** Proc. Imper. Acad. Japan, *16*, 1940, 299, *17*, 1941, 38. — 11. **Zeile, K.,** Zeitschr. f. Vitamin-, Hormon- u. Fermentforsch., *3*, 1949/1950, 540.

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# An antibiotic agent from *Marasmius graminum*

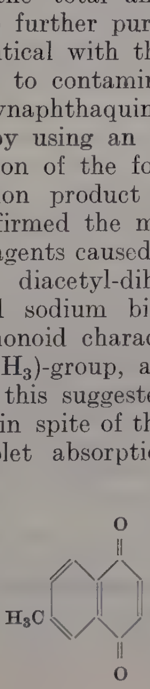
By GERD BENDZ

With 1 figure in the text

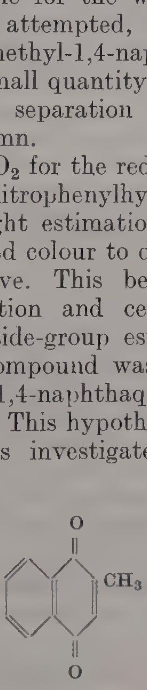
It has been reported earlier that species of the genus *Marasmius* produce substances inhibiting the growth of *Staphylococcus aureus* (1). The culture medium of *Marasmius graminum* obtained from the Institute of Physiological Botany, University of Uppsala, has been the subject of a chemical investigation, and it has been found, that it contains several agents active against *Staphylococcus aureus*. In two preliminary communications the isolation of one of these agents and its identification as 6-methyl-1,4-naphthaquinone has been reported (2), (3).

The quinone was first isolated as a red pigment which after steam distillation and repeated crystallizations from dilute alcohol, dilute acetic acid or petroleum ether, had a constant m.p. of 87°–88° C, and bore every evidence of being homogeneous. As the total amount available for the whole investigation was less than 1 gm, no further purification was attempted, until the pigment was proved to be identical with the yellow 6-methyl-1,4-naphthaquinone (I). The red colour was due to contamination by a small quantity of a similar substance, probably a hydroxynaphthaquinone, and the separation of the two compounds was accomplished by using an alumina column.

The first indication of the formula $C_{11}H_8O_2$ for the red pigment was obtained when a condensation product with 2,4-dinitrophenylhydrazine was analyzed, and this value confirmed the molecular weight estimations made by the RAST method. Reducing agents caused the yellow-red colour to disappear and reductive acetylation gave a diacetyl-dihydro-derivative. This behavior, as well as the insolubility in cold sodium bisulphite solution and certain colour reactions indicated an α-quinonoid character. Methyl-side-group estimation gave evidence of at least one $C(CH_3)$-group, and a leuco-compound was obtained on catalytic hydrogenation. All this suggested a methyl-1,4-naphthaquinone structure to be the most probable in spite of the red colour. This hypothesis was also confirmed when the ultra-violet absorption curve was investigated and compared with



(I)



(II)

that of 2-methyl-1,4-naphthaquinone (II). The spectrum of the red pigment had all the characteristic features which may be regarded as typical of the 1,4-naphthaquinone structure and was similar in many ways to the spectrum of 2-methyl-naphthaquinone. The pigment was oxidized with potassium permanganate to an acid, which on isolation and identification proved to be trimellitic acid. This established the 6-position of the methyl group.

The synthesis of 6-methyl-1,4-naphthaquinone is briefly described in a patent specification (4) but no melting point was given. The quinone was synthesized and melted after several recrystallizations at 90°–91° C and at 87°–88° C when mixed with the red crystals. The ultra-violet absorption curves of the two compounds were identical in the region of 2400–2600 Å, whereas a small but obvious difference was noticed in the region 2600–2850 Å.

After the chromatographic purification of the red pigment, in which great difficulties were encountered, a yellow substance was obtained. It was recrystallized, and proved by mixed melting point, analysis and absorption spectrum to be identical with the synthetic sample of 6-methyl-1,4-naphthaquinone.

Derivatives of α-naphthaquinones with a methyl group in the 2-position have been thoroughly investigated with regard to their biological activity, and it has been found that they are capable, for example, of influencing respiratory processes in certain bacteria (5) and of inhibiting some enzymes such as yeast carboxylas (6). As far as the author knows, no biological investigations have ever been made on naphthaquinone derivatives with a methyl group in the benzenoid ring, but as most quinones, as well as their reduced forms, exhibit biological activity, it is not surprising to find, that not only 6-methyl-naphthaquinone and the corresponding hydroquinone, but also the intermediary products formed when synthesizing this quinone viz. "isoprene-quinone" and 6-methyl-5,8-dihydro-1,4-naphthahydroquinone are also active against *Staphylococcus aureus*. A report of the antibacterial actions of these quinones will be published later elsewhere.

## Experimental[1]

**Cultural conditions.** The culture solution used was a modification of the Czapek-Dox medium with addition of aneurin and one per cent malt extract. After ten days of growth at 25° C the mycelium starts the antibiotic production; the activity curve attains a maximum after one or two days and then declines rapidly. The filtered substrate contained nearly all the active material and only small traces have been found in the mycelium.

During the isolation of the quinone, the other antibiotic agents which are soluble in ether and not volatile with steam, are partly destroyed. It is possible, that one of them is identical with 6-methyl-1,4-naphthahydroquinone which shows good biological activity, as a small quantity of the red pigment was obtained when the residue, left after evaporation of an ether solution, was treated with ferric chloride and steam distilled.

**Isolation and purification of 6-methyl-1,4-naphthaquinone.** The filtered culture solution was distilled in vacuum and a yellow, aqueous distillate collected.

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[1] The melting points given herein are uncorrected.

The distillation was carried out while keeping the volume in the distillation flask constant by water addition, until the distillate was colourless. Extraction of the distillate with chloroform, which was dried over anhydrous sodium sulphate and evaporated under reduced pressure, left a red residue. This was subjected to steam distillation at ordinary pressure and after cooling, the red pigment was collected from the distillate as small red needles m.p. 86°–88° C. The melting point varied slightly from batch to batch. After three recrystallizations from petroleum ether the m.p. was constant 87°–88° C.

Found: average value C 75.63 H 4.71 $C(CH_3)$ 5.63 M (Rast) 173.

The substance was readily soluble in nearly all organic solvents, less soluble, however, in ligroin and petroleum ether and only sparingly in water. Its solutions were yellow with a shade of red. If alkali was added to an aqueous solution a violet colour was formed which turned brown on standing; on acidification a dark red precipitate was formed. The pigment dissolved in concentrated sulphuric acid with a wine red, slowly darkening colour and when treated with ethyl cyano acetate and ammonia gave a violet blue colour (7). In acetone it decolourised potassium permanganate instantaneously and was reduced by stannous chloride in hydrochloric acid and by zinc in sulphuric acid forming a dark brown quinhydrone.

For further purification of the red pigment chromatographic trials were made with many different absorbents and various solvents but it was impossible to detect any inhomogenity of the pigment with any combination but the following. Aluminium oxide standardised according to BROCKMANN washed with 1 N hydrochloric acid (8), air-dried, washed with methanol and again air-dried was used as absorbent. All coloured material was absorbed from a ligroin solution and on elution with alcohol a yellow-green band passed through the column. The remaining dark red substance was eluted with aqueous methanol containing acetic acid. Only a very small amount thereof has yet been obtained, some of its properties might indicate a hydroxynaphthaquinone structure, such as the red violet colour in sodium hydroxide solution. Evaporation of the yellow-green percolate left a yellow-brown partly crystalline residue which after several recrystallizations yielded golden, yellow needles with a constant melting point of 90°–91° C.

$C_{11}H_8O_2$ (172.17) Calc. C 76.73 H 4.68
Found C 76.39 H 4.50

The mixed melting point with 6-methyl-1,4-naphthaquinone showed no depression and the isolated compound was identical in all properties with the synthetic quinone.

**Acetylation of the red pigment.** When zinc dust (0.1 gm) was added to a solution of the pigment (80 mg) in a mixture (1 c.c.) of acetic anhydride and pyridine (20: 1) heat was evolved and a pale yellow solution was formed. The colourless crystalline precipitate, obtained on pouring it into cold water, was collected and crystallized several times from dilute alcohol. M.p. 102°–103° C.

$C_{15}H_{14}O_4$ Calc. C 69.76 H 5.46 M 258.26
Found C 69.48 H 5.36 M (Rast) 250

The diacetyl-dihydro-derivative of 6-methyl-naphthaquinone synthesized in the same way melted at 102°–102.5° C and the mixed melting point was 102°–103° C.

**Catalytic hydrogenation.** A solution of the pigment (37.3 mg) in alcohol (10 c.c.) was shaken with hydrogen in presence of platinum black (10 mg). Absorption of 5.2 c.c. hydrogen was very rapid, whereafter no appreciable amount was more absorbed (calc. for 1 mol $H_2$ 4.85 c.c.). The solution was colourless but turned yellow-red in the air. After filtration and evaporation in vacuum a dark brown residue was left, which after treatment with ferric chloride was steam distilled. Red needles were obtained in the distillate, m.p. and mixed m.p. with the pigment was 85°–86° C.

**Condensation with 2,4-dinitrophenylhydrazine.** When the pigment (30.2 mg) was added to a solution of 2.4-dinitrophenylhydrazine (50 mg) in alcohol and concentrated hydrochloric acid (2 c.c.) (20: 1) heat was evolved and a thick red brown precipitate was almost immediately formed. This was filtered and crystallized from toluene and was then obtained as brick-red needles which were dried over phosphorous pentoxide in high vacuum at 100° C for several hours. When heated in a capillary tube the compound gradually darkened. At 253° C it was nearly black but had not melted.

| | | | | |
|---|---|---|---|---|
| $C_{17}H_{12}O_5N_4$ | Calc. | C 57.95 | H 3.43 | N 15.91 |
| | Found | C 58.34 | H 3.51 | N 16.07 |

The same compound was also obtained from 6-methyl-naphthaquinone and no difference could be observed when heating the synthetic sample and a mixture of the two compounds in capillary tubes.

**Oxidation.** A solution of the pigment (75 mg) and potassium permanganate (0.5 gm) in water (15 c.c.) was heated under reflux for five hours. The cooled solution was decolourized by means of methanol, acidified, filtered and concentrated in vacuum to a small volume. This solution was repeatedly extracted with ether which on evaporation left a colourless residue. After several crystallizations from water, needles were obtained m.p. 213°–216° C (decomp.). A small sample of the acid further purified by precipitation from an acetone-benzene solution was heated in a small flask in vacuum at 250° C. A colourless sublimate was formed m.p. 164° C, a mixture with trimellitic anhydride (9) melted at 162°–164° C.

Electrometric titration:

10.19 mg acid required 1.46 c.c. 0.0986 $N$ NaOH Eq.-w 70.79

| | | | | |
|---|---|---|---|---|
| $C_9H_6O_6$ | Calc. | C 51.44 | H 2.88 | Eq.-w 70.04 |
| | Found | C 51.18 | H 2.90 | Eq.-w 70.79 |

**Synthesis of 6-methyl-1,4-naphthaquinone.** A mixture of p-benzoquinone (12 gm), isoprene (10 gm) and benzene (20 c.c.) in a flask closed with a wired-on rubber stopper was heated to 55–60° C for fourteen hours. The solution was filtered, petroleum ether (40 c.c.) was added and after cooling a heavy precipitate was

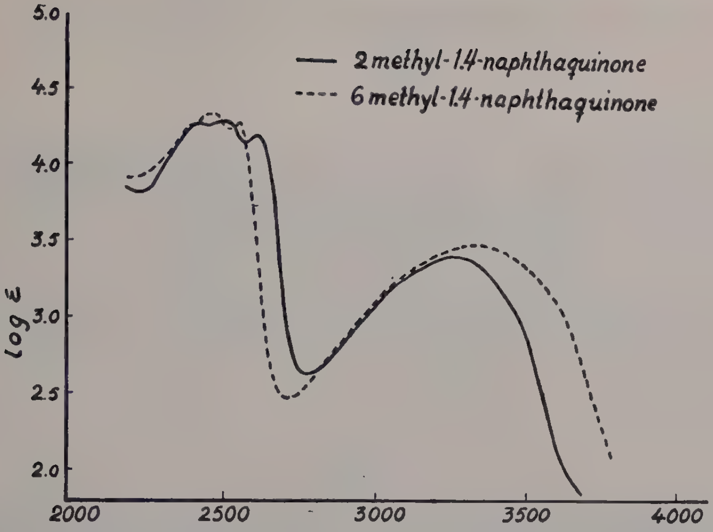

Fig. 1.

formed. After crystallization from petroleum ether the "isoprene-quinone" melted at 84°–85° C. The synthesis of this product has also been described by EULER et al. (10). For isomerisation to 6-methyl-5,8-dihydro-1,4-naphthahydroquinone the directions given in a patent specification (4) were used. A mixture of water (25 c.c.) and concentrated hydrochloric acid (25 c.c.) was added to a warm solution of the "isoprene-quinone" (5 gm) dissolved in alcohol (40 c.c.). After cooling, the reaction mixture was diluted with water (300 c.c.). The precipitate was filtered by suction, washed until neutral and crystallized from carbon tetrachloride. It was obtained as small colourless needles melting at 169°–170° C. The oxidation of this product to the quinone was performed in acetic acid first with sodium nitrite and then sodium dichromate in sulphuric acid as used by L. F. FIESER (11) for 1,4-naphthaquinone. The 6-methyl-quinone could also be obtained by oxidation of the "isoprene-quinone" in acetic acid with sodium dichromate according to L. F. FIESER (11). After four crystallizations from dilute acetic acid the quinone was obtained as golden yellow needles melting at 90°–91° C.

**6-methyl-1,4-naphthahydroquinone** was obtained by reduction of the quinone in alcohol with stannous chloride in concentrated hydrochloric acid. After crystallization from dilute alcohol it was obtained as colourless needles m.p. 165°–167° C.

**Ultra-violet absorption measurements.** 2-methyl-naphthaquinone was obtained by oxidation of β-methyl-naphthalene, m.p. 104°–105° C. The absorption spectra of the two quinones (fig. 1.) were measured in a Beckman spectrophotometer, the solvent was 2,2,4-trimethylpentane. 6-methyl-naphthaquinone exhibits the following maxima and log ε values

| | | | |
|---|---|---|---|
| λ max | 2480 Å | log ε | 4.35 |
| | 2560 Å | | 4.28 |
| | 3350 Å | | 3.49 |

The intention was to measure also the absorption spectrum of 5-methyl-naphthaquinone in order to compare the three isomeric quinone absorption curves. 5-methyl-naphthaquinone is described as yellow needles m.p. 102–103° C (12) obtainable on oxidation of α-methyl-naphthalene with chromic acid, and its structure was proved by degradation to hemimellitic acid. The quinone was synthesized accordingly, the absorption spectrum was, however, identical with that of 2-methyl-naphthaquinone and the melting point was not depressed on admixture with this quinone. Another α-naphthaquinone viz. 1-methyl-5,8-naphthaquinone is also described (13) with a m.p. of 121–122° C. Probably this is the true 5-methyl-naphthaquinone. This investigation is in progress.

## SUMMARY

An antibiotic agent produced by *Marasmius graminum* has been isolated as a red crystalline compound. After being further purified chromatographically, it was finally obtained as a yellow substance and identified by derivatives and degradation to trimellitic acid, as 6-methyl-1,4-naphthaquinone. The ultraviolet absorption spectra of 2- and 6-methyl-naphthaquinones have been measured.

### Acknowledgements

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REFERENCES. 1. **Melin, E., Wikén, T.** and **Öblom, K.**, Nature *159*, 840 (1947). — 2. **Bendz, G.**, Acta Chem. Scand. *2*, 192 (1948). — 3. **Bendz, G.**, Acta Chem. Scand. in press. — 4. **I. G. Farbenindustrie Akt.-Ges.** E. P. 324.661, Chem. Zentr. 1930 II, 809. — 5. **Spruit, C. J. P.** and **Schuiling, A. L.**, Rec. trav. chim. *64*, 219 (1945). — 6. **Kuhn, R.** and **Beinert, H.**, Ber. *80*, 101 (1947) — 7. **Craven, R.**, J. Chem. Soc. *1931*, 1605. — 8. **Wieland, T.**, Hoppe-Seyler's Z. physiol. Chemie *273*, 24 (1942). — 9. **Schultze, W.**, Ann. *359*, 129 (1908). — 10. **v. Euler, H., Hasselquist, H.** and **Glaser, A.**, Arkiv för Kemi *3*, nr 2 (1951). — 11. **Fieser, L. F.**, J. Am. Chem. Soc. *70*, 3168 (1948). — 12. **Herzenberg, J.** and **Ruhemann, S.**, Ber. *60*, 889 (1927). — 13. **Veselý, V., Štursa, F., Olejnicek, H.** and **Rein, E.**, Collect. Trav. chim. Tchecoslovaquie I, 493 (1929). Chem. Zentr. 1930 I. 2734.

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# Acetic acid metabolism in *Rhodospirillum rubrum* under anaerobic condition. III.

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In two previous papers (1, 2) we have described the cultivation of *Rhodospirillum rubrum* under anaerobic conditions and light exposure using acetate and bicarbonate as the main sources of carbon. By using $C^{13}$- and $C^{14}$-labelling of the different carbon atoms of the substrate (series I: $C^{13}H_3C^{14}OONa$, $NaHCO_3$ and series II: $CH_3COONa$, $NaHC^{14}O_3$) and subsequent isolation of alanine, aspartic acid, and glutamic acid from the proteins of the two series, we were able to trace the connection between acetate metabolism and the photosynthetic utilization of $CO_2$. This paper gives the main outlines of the distribution of $C^{13}$ and $C^{14}$ from the substrate in different carbon atoms of glycine, serine, valine, leucine, isoleucine, threonine and the basic and aromatic amino acids, from the same cultivation series as presented in part II of this investigation (2). The isolation procedures followed the methods previously described by us (3, 4, 5). The degradation methods were likewise described in these papers, with the exception of the isolation of the terminal carbon atoms of valine and leucine. This procedure followed the isolation of acetone as outlined by BLOCK and BOLLING and COOK (6, 7).

Valine and leucine were both degraded by oxidizing to acetone with permanganate by the "aeration" method. From one half of the acetone solution the acetone was isolated as its Hg-complex. The precipitate was dried over potassium hydroxide; for combustion it was placed in a Pt-vessel on a piece of ice and dissolved in a few drops of conc. HCl, followed by some drops of water. This solution was then carefully combusted, care being taken not to volatilize the dry residue.

In the other half of the acetone-solution the acetone was converted into iodoform. In order to avoid contamination with foreign carbon (carbonate appears to be especially harmful) the solution was made alkaline with an equal volume of cold, saturated barium hydroxide, filtered if necessary, solid iodine in moderate excess added, and the solution stirred with a mercury-sealed stirrer until the precipitation of iodoform was completed. The iodoform was allowed to settle and washed by decantation several times; finally the suspension was made acid to dissolve any carbonate present and the iodoform filtred off.

In order to test the degradation procedure a sample of acetic acid with 4090 counts per minute of $C^{14}$ in the carboxyl group and 0.455 per cent excess $C^{13}$ in the methyl group, as determined by direct combustion, was converted into lithium acetate and subjected to pyrolysis. The acetone so obtained was degraded as above; the activity was 1340 counts per minute as compared with the theoretical value 1363, which is within the limits of error. The excess $C^{13}$ was 0.270 as compared with the theoretical 0.305; the required factor accordingly 1.13. The combustion of the dry acetone-Hg-complex invariably gave low results (60—70 per cent of the theoretical) which is the reason for the "wet" procedure outlined above.

The iodoform showed an excess of $C^{13}$ = 0.415 per cent, which requires the factor 1.1 to yield the calculated 0.455. (Because of the factors necessary to get the correct amount of isotope content in the control experiment, it is apparent that the degradation procedures of acetone are not fully satisfactory. Work is carried out to improve the methods.)

The results of all these degradations, and the subsequent determinations of the isotope content in different carbon atoms of the amino acid structures are presented in Tables I, II and III.

## Discussion

Our results from the degradation of alanine, aspartic acid, and glutamic acid, described in part II of this series (2), showed that the α-carboxyls of these amino acids were derived mainly from the bicarbonate carbon ("$CO_2$ -carbon"). The α-carbon atoms of alanine and aspartic acid originated from the carboxyl of acetic acid, the β-carbon atoms of these amino acids emanating from the methyl part of the acetate. This, together with the finding that the γ-carboxyl of glutamic acid was derived entirely from acetate carboxyl, pointed to that the primary fixation of $CO_2$ occured as a condensation with a 2-carbon structure, originating from acetate, forming a 3-carbon structure with the sequence $C^M—C^C—C^B$ (M indicating to the main origin from the methyl group of acetate, C origin from the carboxyl, B from bicarbonate carbon — $CO_2$-carbon). This sequence, which was observed in alanine, should indicate the same order in pyruvic acid. The sequence $HOOC^B—C^MH_2—C^C(NH_2)—C^BOOH$, observed in aspartic acid, was in accordance with the assumption of a secondary fixation of $CO_2$ to pyruvate, possibly via a WOOD-WERKMAN-reaction (8). That the α- and the γ-carboxyls of glutamic acid (and the corresponding α-ketoacid, α-ketoglutaric acid) should be derived, as observed, from the $CO_2$-carbon and the acetate carboxyl, respectively, could consequently be explained on the basis of an acetate-oxalacetate condensation as outlined by KREBS and WOOD (9 10).

As shown by Table I the isotope distribution in serine was analogous to that of alanine, indicating a close connection between these two amino acids and pyruvate. Since the origin of the carbon atoms of serine, as pictured in the sequence $C^MH_2OH—C^CH(NH_2)—C^BOOH$ was: the β-carbon atom from $CH_3$, the α-atom from COOH and the carboxyl from $CO_2$, it was to be expected that glycine should originate from the acetate carboxyl and $CO_2$, as the result of a formate split of serine (11). This is in fact the case, as shown by the data from Table I.

When comparing the labelling of the closely related aspartic acid, alanine, serine and glycine it is evident that the formation of these carbon stuctures is based on the same principle i.e. the carboxyl emanating from $CO_2$ -carbon,

*Table I*

Isotope content of different carbon atoms of glycine, serine and threonine, isolated from *Rhodospirillum rubrum*, cultivated on acetate and bicarbonate, labelled according to series I and II. $C^{13}$ is given in atom per cent excess, $C^{14}$ in counts per minute per 15 mg of barium carbonate.

| | Series I | | Series II |
|---|---|---|---|
| | $C^{13}$ | $C^{14}$ | $C^{14}$ |
| *Substrate :* | | | |
| Series I, $C^{13}H_3C^{14}OONa$ | 3.55 | 26300 | — |
| $NaHCO_3$ | — | — | — |
| Series II, $CH_3COONa$ | — | — | — |
| $NaHC^{14}O_3$ | — | — | 26100 |
| Glycine, carboxyl (by ninhydrin) | 0.035 | 411 | 1735 |
| » , total combustion | 0.055 | 1218 | 858 |
| *Compiled values :* | | | |
| Carboxyl | 0.035 ± .005 | 411 ± 15 | 1735 ± 55 |
| α-carbon atom | 0.075 ± .01 | 2025 ± 80 | 19 |
| Serine (as alanine) total combustion | 0.17 | 774 | 974 |
| » , carboxyl (by ninhydrin) | 0.04 | 390 | 2840 |
| » , β-carbon atom as iodoform | 0.21 | 140 | 62 |
| *Compiled values :* | | | |
| Carboxyl | 0.04 ± .005 | 390 ± 20 | 2840 ± 85 |
| α-carbon atom | 0.24 | 1792 ± 70 | 20 ± 20 |
| β-carbon atom | 0.21 | 140 ± 15 | 62 ± 12 |
| Threonine, total combustion | 0.125 | 641 | 910 |
| » , carboxyl (by ninhydrin) | 0.035 | 396 | 1280 |
| » , β- and γ-carbon atoms by combustion of acetaldehyde-semi-thio-carbazone | 0.12 | 150 | 680 |
| » , γ-carbon atom as iodoform | 0.035 | 410 | 1505 |
| *Compiled values :* | | | |
| Carboxyl | 0.035 ± .005 | 396 ± 20 | 1280 ± 40 |
| α-carbon atom | 0.23 ± .04 | 1850 ± 300 | 930 |
| β-carbon atom | 0.21 ± .02 | 0 | 0 |
| γ-carbon atom | 0.035 ± .01 | 410 ± 35 | 1505 ± 150 |

the α-carbon atom from the carboxyl of acetate, the β-carbon atom from the methyl group of acetate (in 3- and 4-carbon stuctures). From the values obtained from threonine it is shown that this principle holds even for that amino acid. Furthermore, our results from the degradation of the rest of the isolated amino acids indicate that in all cases, but three, the bicarbonate-$C^{14}$ is the main source of the amino acid carboxyls, the exceptions being the leucine, phenylalanine and histidine carboxyls. The two former, containing a very low amount of bicarbonate-$C^{14}$ (Tables II and III), are derived from the acetate carboxyl. The histidine carboxyl, on the contrary, has the highest $C^{13}$-content of all amino acid carboxyls investigated, but a low $C^{14}$-content from the acetate carboxyl, and is therefore most probably derived from the methyl group of acetate. It is interesting to note that the origin of the three amino acid carboxyls mentioned seem to be the same in the anaerobic *Rhodospirillum* as in the aerobic *Torulopsis* and *E. coli*, previously investigated (4, 5).

The labelling of threonine has the sequence $C^BH_3—C^MHOH—C^{CM}H(NH_2)—C^BOOH$, indicating that the bicarbonate-carbon is responsible for the carboxyl *and* the γ-carbon atom, the carboxyl of acetate being the main source of the α-carbon. The methyl group of acetate appears predominantly in the β- and α-positions. The whole picture ressembles that of aspartic acid (2); the similarity of the labellings from acetate in aerobic system of threonine and aspartic acid has been pointed out by us in some earlier papers. (4, 5, 12). Our findings in *Rhodospirillum* concerning the labelling of threonine gives additional support to the view that aspartic acid might be the precursor of this amino acid, possibly via homoserine.

With regard to the three terminal carbon atoms in valine and leucine, these are in aerobic systems entirely derived from the methyl group of acetate. In *Rhodospirillum*, however, we find that acetone, obtained from the γ—β—γ-carbon atoms of valine by oxidation, contains a considerable amount of $C^{14}$, derived from the acetate carboxyl. This $C^{14}$-content is localized to the β-atom, the terminal methyl groups being $C^{14}$-free and containing $C^{13}$ (from the acetate methyl group). By calculation from the isotope values of acetone and the iso-butyraldehyde-2-4-dinitro-phenylhydrazone (by combustion), the isotope content of the α-carbon atom could be estimated. As the result of these calculations the whole sequence of the labelled carbon atoms of valine was obtained, showing the picture $(C^MH_3)_2—C^CH—C^CH(NH_2)—C^BOOH$ (Table II). It is very difficult to conceive a metabolic scheme, by which this type of labelling (and thus the formation of valine) could be reconciled. That the three terminal carbon atoms contain the isotope sequence $C^{13}—C^{14}—C^{13}$ (contrary to the aerobic $C^{13}—C^{13}—C^{13}$) is not too unexpected, in view of the unlikeliness of a $C^{13}—C^{13}—C^{13}$-sequence to be found in *Rhodospirillum*. Since so many principally analogous metabolic features are observed in *Rhodospirillum* as compared with aerobic systems (*E. coli*, *Torulopsis*) we suggest that the terminal part of valine, labelled $C^{13}—C^{14}—C^{13}$ originates from the same structure, which in aerobic systems has the labelling $C^{13}—C^{13}—C^{13}$. The three non-carboxylic carbon atoms in glutamic acid, for example, would meet that requirement, and consequently the corresponding carbon atoms of the pre-stages of α-ketoglutaric acid, citric acid a.o. Since the labelling of the α-carbon and the carboxyl of valine is of the apparent "normal" type, as found in *Rhodospirillum* in many other amino acids, we might, with due caution, imagine the valine structure as obtained from citrate and glycine, the three non-carboxylic atoms of citrate forming the three terminal carbon atoms of valine.

The low over-all isotope content of leucine makes it somewhat difficult to certify, whether the relatively small $C^{14}$-content (of carboxyl origin) in the acetone (obtained by oxidation) is significant or not. However, by applying the same reasoning as in the case of valine, any plain $C^{13}—C^{13}—C^{13}$ configuration would be very improbable in *Rhodospirillum*. Thus the 90 counts per minute, as calculated for the γ-carbon atom could, most probably, be regarded as a positive indication for a $C^{13}—C^{14}—C^{13}$ sequence in the terminal part of the

*Table II*

Isotope content of different carbon atoms of valine, leucine and isoleucine isolated from *Rhodospirillum rubrum*, cultivated on acetate and bicarbonate, labelled according to series I and II. $C^{13}$ is given in atom per cent excess, $C^{14}$ in counts per minute per 15 mg of bazium cazbonete.

| | Series I | | Series II |
|---|---|---|---|
| | $C^{13}$ | $C^{14}$ | $C^{14}$ |
| *Substrate :* | | | |
| Series I, $C^{13}H_3C^{14}OONa$ | 3.55 | 26300 | — |
| $NaHCO_3$ | — | — | — |
| Series II, $CH_3COONa$ | — | — | — |
| $NaHC^{14}O_3$ | — | — | 26100 |
| Valine, total combustion | 0.09 | 447 | 128 |
| » , carboxyl (by ninhydrin) | 0.025 | 79 | 584 |
| » , α-, β-, γ-, and γ′-carbon atoms, mean value, by combustion of isobutyraldehyde-2.4-di-nitro-phenyl-hydrazone | 0.10 | 532 | 13 |
| » , β-, γ-, and γ′-carbon atoms, mean value, by combustion of acetone Hg-complex | 0.12 | 494 | 4 |
| » , γ-, and γ′-carbon atoms, mean value, as iodoform | 0.16 | 24 | 4 |
| *Compiled values :* | | | |
| Carboxyl | 0.025 ± .005 | 79 ± 8 | 584 ± 20 |
| α-carbon atom | 0.04 ± .02 | 700 ± 70 | 30 |
| β-carbon atom | 0.04 ± .02 | 1428 ± 45 | 4 ± 4 |
| γ- and γ′-carbon atoms | 0.16 ± .01 | 24 ± 6 | 4 ± 2 |
| Leucine, total combustion | 0.02 | 82 | 30 |
| » , carboxyl (by ninhydrin) | 0.005 | 333 | 31 |
| » , α-, β-, γ-, δ-, and δ′-carbon atoms, mean value, by combustion of isovaleraldehyde-2.4-di-nitro-phenyl-hydrazone | 0.04 | 47 | 12 |
| » , γ-, δ-, and δ′-carbon atoms, mean value, by combustion of acetone Hg-complex | 0.03 | 38 | 6 |
| » , δ- and δ′-carbon atoms, mean value, as iodoform | 0.03 | 10 | 7 |
| *Compiled values :* | | | |
| Carboxyl | 0.005 ± 005 | 333 ± 15 | 31 ± 6 |
| α- and β-carbon atoms, mean value | 0.04 ± .02 | 121 ± 30 | 22 ± 11 |
| γ-carbon atom | 0.03 ± .01 | 95 ± 20 | 4 ± 4 |
| δ- and δ′-carbon atoms, mean value | 0.03 ± .005 | 10 ± 3 | 7 ± 2 |
| Isoleucine, total combustion | 0.06 | 200 | — |
| » , carboxyl (by ninhydrin) | 0.02 | 209 | — |
| » , α-, β-, γ-, γ′-, δ-carbon atoms, mean value, by combustion of corresponding aldehyde 2.4-di-nitro-phenyl-hydrazone | 0.045 | 95 | — |

*Table III*

Isotope content of different carbon atoms of proline, tyrosine, phenylalanine, arginine, lysine and histidine, isolated from *Rhodospirillum rubrum*, cultivated on acetate and bicarbonate, labelled according to series I and II. $C^{13}$ is given in atom per cent excess, $C^{14}$ in counts per minute per 15 mg of barium carbonate.

| | Series I | | Series II |
|---|---|---|---|
| | $C^{13}$ | $C^{14}$ | $C^{14}$ |
| *Substrate :* | | | |
| Series I, $C^{13}H_3C^{14}OONa$ | 3.55 | 26300 | — |
| $NaHCO_3$ | — | — | — |
| Series II, $CH_3COONa$ | — | — | — |
| $NaHC^{14}O_3$ | — | — | 26100 |
| *Proline*, total combustion | 0.205 | 1270 | 504 |
| » , carboxyl (by ninhydrin) | 0.04 ± .005 | 392 ± 20 | 1732 ± 55 |
| » , ring carbon atoms, mean value | 0.25 | 1490 | 200 |
| *Tyrosine*, total combustion | 0.17 | 941 | 852 |
| » , carboxyl (by ninhydrin) | 0.03 ± .005 | 251 ± 10 | 2710 ± 80 |
| » , α-, β-, carbon atoms and ring, mean value | 0.185 | 1027 | 625 |
| *Phenylalanine*, total combustion | — | — | 21 |
| » , carboxyl (by ninhydrin) | — | — | 33 ± 4 |
| » , α-, β-, carbon atoms and ring, mean value | — | — | 18 |
| *Arginine*, total combustion | 0.075 | 468 | 448 |
| » , carboxyl (by ninhydrin) | 0.035 ± .005 | 224 ± 7 | 676 ± 25 |
| » , guanido-carbon | 0.035 ± .01 | 550 ± 110 | 2000 ± 20 |
| » , α-, β-, γ-, and δ-, carbon atoms, mean value | 0.10 | 505 | 0 |
| » , as ornithine, total combustion | 0.09 | 408 | 78 |
| *Lysine*, total combustion | 0.12 | 634 | 514 |
| » , carboxyl (by ninhydrin) | 0.035 ± .005 | 295 ± 10 | 1322 ± 55 |
| » , α-, β-, γ-, δ-, and ε-, carbon atoms, mean value | 0.14 | 722 | 353 |
| *Histidine*, total combustion | 0.07 | 279 | 118 |
| » , carboxyl (by ninhydrin) | 0.07 ± .01 | 45 ± 7 | 60 ± 10 |
| α-, β-, and ring carbon atoms, mean value | 0.07 | 326 | 130 |

*Rhodospirillum*-leucine. As to the rest of the molecule, it is interesting to denote that $CO_2$-carbon has *not* contributed to any part of the leucine carbon atoms, all of them, including the carboxyl, being derived from acetate carbon. The parallel with the aerobic *E. coli* and *Torulopsis* is striking: the carboxyl of leucine from these organisms is derived directly from the acetate carboxyl, *not* via the pathway over secondary fixation of respiratory $CO_2$. (4, 5).

The case of proline is interesting. Wheras the carboxyl is prodominantly derived from bicarbonate-carbon, the contribution of this carbon source to the pyrrolidine structure is low in comparison to that of the acetate carbon. Comparing

*Table IV*

Survey of the isotope content of 15 amino acid carboxyls from *Rhodospirillum rubrum* compiled from Tables I—III, and Table I in part II of this investigation (2). $C^{13}$ is given in atom per cent excess, $C^{14}$ (of series I and II) in counts per minute per 15 mg of barium carbonate.

$'C^{14B}$, $'C^{14C}$ and $'C^{13M}$ denote isotope content in per cent of corresponding content in the bicarbonate-carbon, acetate carboxyl and acetate methyl group as present in the medium at the start of the experiment.

| | Series I | | Series II | | | | | |
|---|---|---|---|---|---|---|---|---|
| (*Substrate*) | $C^{13M}$ | $C^{14C}$ | $C^{14B}$ | $'C^{13M}$ | $'C^{14C}$ | $'C^{14B}$ | $'C^{14B} : 'C^{14C}$ | $'C^{14C} : 'C^{13M}$ |
| Bicarbonate carbon ...... | — | — | 26100 | | | 100 | | |
| Acetate carboxyl......... | — | 26300 | — | | 100 | | | |
| Acetate methyl .......... | 3.55 | — | — | 100 | | | | |
| 1. Glut. acid- γ- COOH | 0.005 | 5000 | 190 | 0.14 | 19.0 | 0.74 | 0.04 | 136 |
| 2. » - α- COOH | 0.055 | 580 | 3035 | 1.55 | 2.2 | 11.6 | 5.3 | 1.42 |
| 3. Aspartic- α- COOH | 0.08 | 780 | 2300 | 2.25 | 2.95 | 8.8 | 3.0 | 1.32 |
| 4. » - β- COOH | 0.03 | 730 | 3000 | 0.85 | 2.8 | 11.5 | 4.1 | 3.30 |
| 5. Alanine- COOH | 0.025 | 210 | 2350 | 0.7 | 0.8 | 9.0 | 11.3 | 1.14 |
| 6. Serine- COOH | 0.04 | 390 | 2840 | 1.1 | 1.5 | 10.9 | 7.3 | 1.36 |
| 7. Glycine- COOH | 0.035 | 411 | 1735 | 0.99 | 1.56 | 6.65 | 4.3 | 1.58 |
| 8. Valine- COOH | 0.025 | 79 | 584 | 0.7 | 0.3 | 2.25 | 7.5 | 0.43 |
| 9. Leucine- COOH | 0.005 | 333 | 31 | 0.14 | 1.26 | 0.12 | 0.1 | 9.0 |
| 10. Isoleucine- COOH | 0.02 | 209 | — | 0.56 | 0.8 | — | — | 1.42 |
| 11. Threonine- COOH | 0.035 | 396 | 1280 | 0.99 | 1.5 | 4.9 | 3.3 | 1.52 |
| 12. Tyrosine- COOH | 0.03 | 251 | 2710 | 0.85 | 0.96 | 10.2 | 10.6 | 1.13 |
| 13. Phenylalanine- COOH | — | — | 21 | — | — | 0.1 | — | — |
| 14. Proline- COOH | 0.04 | 392 | 1732 | 1.1 | 1,5 | 6.65 | 4.4 | 1.36 |
| 15. Lysine- COOH | 0.035 | 295 | 1322 | 0.99 | 1.12 | 5.1 | 4.5 | 0.14 |
| 16. Arginine- COOH | 0.035 | 224 | 676 | 0.99 | 0.85 | 2.6 | 3.1 | 0.87 |
| 17. Histidine- COOH | 0.07 | 45 | 60 | 1.97 | 0.17 | 0.23 | 1.15 | 0.08 |
| Mean value (1, 9, 10, 13, 17 not included) | | | | | | | 5.7 | 1.50 |
| 18. Resp. $CO_2$ at the end of exp. in medium... | 0.075 | 1035 | 26000 | 2.12 | 3.9 | 99.0 | 25.4 | 1.85 |
| *For comparison:* | | | | | | | | |
| 19. Threonine γ-C atom .. | 0.035 | 410 | 1505 | 0.99 | 1.56 | 5.8 | 3.7 | 1.58 |
| 20. Arginine guanido-C .. | 0.035 | 550 | 2000 | 0.99 | 2.1 | 7.6 | 3.6 | 2.12 |

the contributions of bicarbonate-carbon, acetate carboxyl-carbon and methyl-carbon (in per cent of the corresponding isotope content of these sources in the medium) we find a ratio of 0.77 : 1.49 : 7.1 = 1 : 6.4 : 9.2. The corresponding ratio for the side-chain of glutamic acid (calculated from (2), Table III is 1 : 7.4 : 9.1. Thus, it seems probable that there exists a close relationship in *Rhodospirillum* between glutamic acid and proline, which is furthermore supported by a comparison of the ratio of the three carbon sources (as carried out above) in the carboxyls of these amino acids. The proline carboxyl has the ratio 1 : 0.24 : 0, 16; the corresponding ratio for the glutamic acid α-carboxyl being 1 : 0.20 : 0.16.

The case of the basic amino acids appears very complicated. Until a total degradation of these amino acids could be done, it could only be said that in lysine structure the contribution of its six carbon atoms from bicarbonate-carbon, acetate carboxyl and methyl-carbon show a ratio of 1 : 1.2 : 1.7 and that the carboxyl has a corresponding ratio of 1 : 0.21 : 0.18. The bicarbonate-carbon thus contributes to about 70 per cent of the lysine carboxyl carbon, whereas the corresponding contribution to the five side-chain carbon atoms is only 17 per cent; the rest being accounted for by 33 per cent from the acetate carboxyl and 50 per cent from the acetate methyl group.

The carboxyl of histidine seems to be derived mainly from the acetate *methyl* group, an interesting parallel to the similar findings in aerobic systems (4, 5).

In arginine the carboxyl is mainly derived from $CO_2$. By degradation to ornithine a rough check of the isotope content of the guanido-carbon could be made, showing an origin from $CO_2$-carbon and acetate carboxyl carbon in a ratio of about 4 : 1.

## SUMMARY

Twelve amino acids have been isolated from a culture of *Rhodospirillum rubrum,* grown in two series under anaerobic condition under exposure to light, on acetate and bicarbonate as main sources of carbon. In one of the series the acetate was labelled as $C^{13}H_3C^{14}OONa$, the bicarbonate being non-labelled; in the other series the acetate was non-labelled and the bicarbonate labelled as $NaHC^{14}O_3$. The details of the cultivation procedure and the isolation and degradation of alanine, aspartic acid and glutamic acid from the proteins of the two cultivation series have previously been described (2).

The isotope content in the different carbon atoms of the twelve isolated amino acids of both series was determined by degradation.

In addition to the degradation procedures previously worked out (3, 4, 5) a small-scale procedure for isolation of the three terminal carbon atoms of valine and leucine is described.

It was found that in several cases, in glycine, serine, alanine, threonine and aspartic acid, that the labelling pattern showed the same principal features, namely: *the carboxyl groups originating from bicarbonate-carbon* ($CO_2$), *the α-carbon atoms from the acetate carboxyl.* In all of these cases (with the exception of glycine) the β-carbon atoms were derived from the methyl group of acetate. The γ-carbon atom of threonine emanated mainly from the bicarbonate-carbon, indicating a possible connection between this carbon atom and aspartic acid β-carboxyl.

The total picture of amino acid labellings from bicarbonate-carbon, the acetate carboxyl and methyl group, seems to be in accordance with the assumption of Calvin (13) that part of the $CO_2$-carbon enters the system by condensation with a 2-carbon structure of acetate origin. The resulting 3-carbon-compound should be closely connected to pyruvate and consequently to alanine. (This reaction is with certainty the main feature of the photosynthethic process in *Rhodospirillum.*) A second entrance of $CO_2$ is indicated to occur via β-condensation with pyruvate to oxal acetate.

Our findings with respect to the contribution of acetate carbon atoms to the different amino acids structures point to an entrance of acetate into the system

in, at least, three ways. One is, as previously stated, the photodirected condensation with $CO_2$, forming pyruvate, the second is indicated by the labelling of glutamic acid, and consequently of α-ketoglutarate (2), being most likely the consequence of a primary condensation of acetate with oxal acetate. A third way is indicated by the appearance of the acetate carboxyl (instead of $CO_2$!) in the carboxyls of some amino acids, i.e. phenylalanine and leucine.

The main source for the histidine carboxyl is the methyl carbon of acetate, a parallellism to our previous findings in aerobic systems (4, 5,14).

The similar labellings of serine and alanine point to a close connection between these amino acids. Glycine is with certainty produced from serine by a formate split of the serine β-carbon.

Finally, the whole picture of amino acid origins, is in principle the same in *Rhodospirillum* under anaerobic condition as in aerobic systems, the main differences arising as the consequence of acetate entering by photodirected reactions by condensation with $CO_2$.

From the Wenner-Gren Institute for Experimental Biology, University of Stockholm.

LITERATURE. 1. **C. Cutinelli, G. Ehrensvärd** and **L. Reio,** Ark. Kemi, *2,* Nr 21, 357 (1950). — 2. **C. Cutinelli, G. Ehrensvärd, L. Reio, E. Saluste** and **R. Stjernholm,** Ark. Kemi 3, Nr 20, 315 (1951). — 3. **J. Baddiley, G. Ehrensvärd, E. Klein, L. Reio** and **E.Saluste,** J. Biol. Chem. *183,* 777 (1950). — 4. **G. Ehrensvärd, L. Reio, E. Saluste** and **R. Stjernholm,** J. Biol. Chem. *189,* 93 (1951). — 5. **C. Cutinelli, G. Ehrensvärd, L. Reio, E. Saluste** and **R. Stjernholm,** Acta Chem. Scand. *5,* 353 (1951). — 6. **K. J. Block** and **D. Bolling,** The amino acid composition in proteins and foods, Ch, Thomas, Springfield, Ill. (1945) p. 224. — 7. **C. A. Cook** and **A. H. Smith,** J. Biol. Chem. *85,* 251 (1929—30). — 8. **C. H. Werkman** and **H. G. Wood,** Adv. of Enzymology, Vol. II, Interscience Publishers Inc. New York (1949) p. 147. — 9. **H. A. Krebs,** Adv. of Enzymology, Vol. III, Interscience Publishers Inc. New York (1943) p. 191. — 10. **N. Lifson, V. Lorber, W. Sakami** and **H. G. Wood,** J. Biol. Chem. *176,* 1263 (1948). — 11. **W. Sakami,** J. Biol. Chem. *176,* 995 (1948). — 12. **G. Ehrensvärd, L. Reio, E. Saluste** and **R. Stjernholm,** Publikation 1; 7:de Nordiska Kemistmötet, Helsinki (1950. p. 71. — 13 .**M. Calvin,** J. Chem. Ed. *26,*639 (1949). — 14. **G. Ehrensvärd. L. Reio** and **E. Saluste,** Acta Chem. Scand. 3, 645 (1949).

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Uppsala 1951. Almqvist & Wiksells Boktryckeri AB

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# Studies in experimental rheumatism. II.

## Effects of desoxycorticosterone acetate overdosage and of focal streptococcal infection on scorbutic guinea-pigs

## A. Methods

By HANS VON EULER and LEO HELLER

With 2 figures in the text

The experiments which form the subject of this report were designed as a further led in the systematic testing of a hypothesis pertaining to the pathogenetical mechanism and to the reproducibility in animals of the clinical and pathological syndrome of the rheumatic disease, tentatively advanced by us in an earlier paper of this series (1). We expressed there the view, that the consistent failures of innumerable studies in rheumatism, following different, partly very attractive and as it seems theoretically well founded lines of thought in their attempts to reproduce experimentally the well-known picture of human rheumatism (compare e.g. WAKSMAN (2)), can possibly be ascribed to the innately complex nature of the etiological and physiopathological mechanisms, active in the protean syndrome of rheumatism. It seems quite reasonable to assume, that both the typical, full blossom, pathological lesions and the characteristical clinical symptomatology and localization of rheumatic processes can be produced only by a simultaneous, well balanced action of several etiological factors, partly of exo- and partly of endogenous nature.

The summation of the effects of different agents, all of them either wholly inactive or only irregularly active when acting separately, might thus be thought to constitute the condition indispensable for the successful production of the syndrome in question. Eventual variations in the qualitative and quantitative composition of the sum of rheumatogenic stimuli may then explain the existence of the well-known prodigal variety of forms, courses and localizations of rheumatic processes.

The above view seems to receive support in a certain amount of available experimental evidence. The "rheumatogenic" effect of mineralocorticoids, rather weak in normal, healthy animals, can be potentiated by different kinds of stress (e.g. thyreoidectomy) (3), probably through the intermediary of releasing certain physiopathological mechanisms, absent or inhibited in the healthy individual.

A group of American workers (4) recently reported the aggravating effect of desoxycorticosterone acetate (DCA) on the articular lesions in scorbutic guinea-

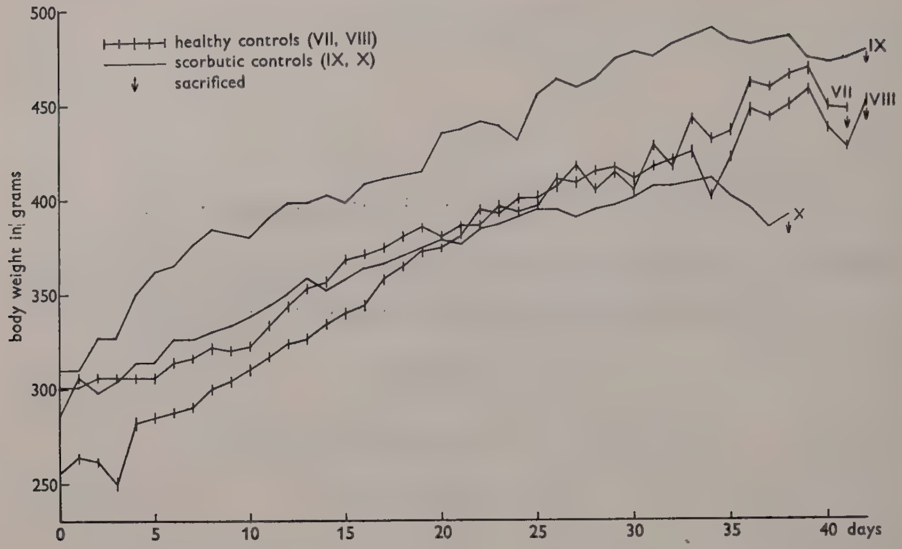

Fig. 1.

pigs. Those observations give a new actuality to the old "scorbutogenic" theory of rheumatism (5), subsequently rejected by its own author (6) because of conflicting experimental evidence. We thought it worth while to repeat the experiments of Schaffenburg et al. (4) and to study the relation of the lesions eventually obtained to those of true rheumatism. In addition, we tested the effect of streptococcal infection, still considered by the majority of rheumatologists to bear some relation to the etiology of the rheumatic disease, on animals subjected to the joint stress of DCA overdosage and vitamin C deficiency. Thus, according to the hypothesis postulated above, three factors suspected to be of some importance in the pathogenesis of rheumatism, were investigated when acting simultaneously upon a group of animals.

## Experimental

### Materials and Methods

*Experiment I,* to be described below, comprized altogether 10 guinea-pigs, all of them females, weighing between 250 and 310 grams (Figs. 1 and 2). Six of them were subjected to a treatment, consisting of simultaneous administration of a dietary regime deficient in vitamin C and of daily injections of high doses of DCA. Two scorbutic controls (Nos. IX and X), receiving no DCA, as well as two healthy controls, whose scorbutigenic diet was supplemented by large doses of fresh orange juice (5 ml daily) given by pipette, served as reference basis for estimating the effects of combined treatment.

The diet used was that described by Dalldorf (7) — the same as in the experiments of Schaffenburg et al. (4). Wishing to produce a rather chronic form of scurvy, to give the arthritic lesions enough time to develop (4), we

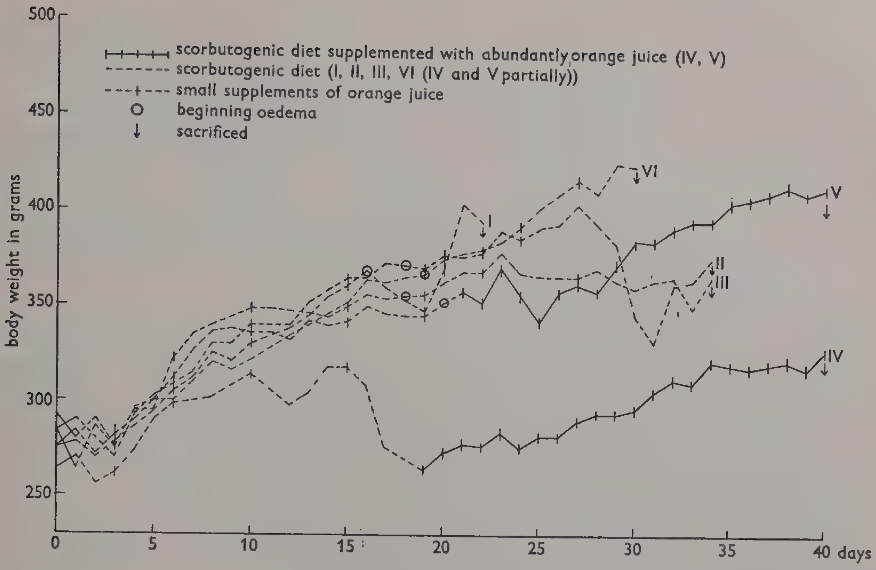

Fig. 2.

supplemented the latter, C-vitamin-free diet by small quantities (1–2 ml) of fresh orange juice, given every 3rd–4th day. Repeated titrations of orange juice samples with Tillmans' reagent (dichlorophenole-indophenole) indicated an average ascorbic acid content of c:a 0.8–1.0 mg/ml. The basal diet, extracted with water and titrated in the same way, was found to contain practically no ascorbic acid. The average daily supply of vitamin C in the animals subjected to the chronically scorbutigenic treatment was thus less than 1 mg/day.

The suspension of crystalline desoxycorticosterone acetate in olive oil was administered subcutaneously. The daily dose of about 5 mg DCA was given in one injection. The DCA treatment was started one day after the beginning of the scorbutigenic treatment. To sensitize the animals to the effects of DCA, all animals (the controls included) were given 1 % sodium chloride solution to drink instead of water (4).

Thus, in the experiment I, we followed fairly exactly the experimental technique of the American workers (4). To make it sure that the effects observed were really due to the combined action of scurvy and DCA, two of the test animals (No:s IV and V), when on the height of their symptoms, got their diet supplemented with abundantly vitamin C (Fig. 2). which resulted in a rapid disappearance of the symptoms, notwithstanding the continued administration of DCA.

The other four test guinea-pigs were sacrificed when on the peak of their symptoms (see below), i.e. after 22–35 days of treatment. The remaining ones, i.e. the controls and those recovered after addition of much orange juice (IV, V), showing no or only very discrete signs of illness, were sacrificed between the 38th and the 42nd day of the experiment.

In a second experiment (see (15) — Table 1.), comprizing a series of 8 animals, all males, body weights ranging from 274 to 361 grams, the effect of a focal streptococcal infection on DCA-treated scorbutic guinea-pigs was investigated. The diet and the dosage of DCA were the same as in Experiment I. Crystalline ascorbic acid was, however, substituted for orange juice to eliminate the possibility of some constituent of orange juice other than ascorbic acid being responsible for the protecting effect of the juice. Ascorbic acid was administered subcutaneously, once daily, in amts. of 15 mg per animal and day, to the 3 animals of the control group (designed by the letter $B$). Two of those animals ($B_1$ and $B_2$) received daily injections of DCA. The 3rd ($B_4$) was spared in quality of healthy control, to test the virulence of the bacterial strain used for healthy guinea-pigs.

The test group, consisting of 5 animals, received both the scorbutigenic diet (7) supplemented by weekly injections of 2 mg of ascorbic acid, given for the 1st time on the 9th day after having started the scorbutogenic diet. The average supply of ascorbic acid acid was thus in that group c:a 0.3 mg — considerably below the normal vitamin C requirements, as given in the literature. All the 5 test animals were daily treated with DCA. The DCA treatment was in that experiment started on the 5th day after the scorbutigenic diet.

On the 15th day of the experiment, two of the test- and one controlanimal ($A_4$, $A_5$, and $B_2$, resp.) received an intramuscular injection of Group C hemolytic streptococci (the streptokinase-producing strain H64 of Cathie (8), obtained through the courtesy of Dr. PER HEDLUND, State Bacteriological Laboratory, Stockholm).

The bacteria were first grown for 18 h. at 37° C in a calf meat trypsin digest medium, then spun down in a laboratory type 'Wifug' angle centrifuge at c:a 2,500 r. p. m., washed free of the medium, resuspended in saline and standardized nephelometrically to contain c:a $5 \times 10^8$ cells/ml. This suspension was then diluted ten times with melted 2 % nutrient agar of c:a 42° C temperature and rapidly injected intramuscularly in the left front paw of the animals, in the proximity of the knee joint, in amounts of 1 ml (containing c:a $5 \times 10^7$ bacterial cells). The addition of agar, by virtue of decreasing the rate of resorption, should help to localize the organisms injected (9), giving a focal infection, more closely reminding us of the situation presumably present in natural streptococcal infections in man, thought to stand in a causal relationship to the human rheumatism.

As no visible symptoms of bacterial generalization (sepsis) followed the injection, two other test-animals ($A_1$ and $A_2$) and the remaining two controls ($B_1$ and $B_4$) were injected 3 days later with a somewhat higher dose of the bacterial suspension (about $2 \times 10^8$ cells) of the same strain. The scorbutigenic and DCA treatments were continued, the animals observed daily for 2 weeks after infection and then challenged by an injection of c:a $10^9$ living H64 bacteria in the right front paw to test an eventually developed hypersensitivity against the streptococcus strain H64. Two animals, one of the test- and one of the control group ($A_5$ and $B_2$, resp.) died, however, spontaneously before the end of the two-weeks' period after the first infection and could thus not be reinfected. At autopsy, guinea-pig $A_5$ showed a heavy diffuse pneumonic infiltration of both lungs and a moderate degree of fatty degeneration of the parenchymatous viscera. There were a few disseminated small haemorrhages in the muscles of the extremities and a well incapsulated little abscess loco injectionis. The joints — also the left knee joint lying in direct neighbourhood of the abscess — showed a wholly normal appearance. May it be anticipated that the latter finding applied to all animals of both experimental series. The death of the control animal $B_2$ was probably due to an emboly of some vital blood vessel, for it occurred suddenly, during a routine DCA injection. No distinct pathological changes in the inner organs or muscles could be found at autopsy by macroscopical examination. The rest of the animals survived the 2nd bacterial injection and were sacrificed between 36 and 49 days after starting the experiment.

All animals (in both experiments) were examined and weighed daily and the body weights recorded. In experiment I, the anteroposterior and lateral diameters of the right paw at the height of the joint formed by the shin and carpal bones were measured at a few days intervals, as done by SCHAFFENBURG et al. (4).

Special attention was paid to the passive and active mobility of all limbs and joints, to the muscle tonus and to the appearance of diffuse or localized swelling or tenderness, recognizable by palpation.

The animals were killed by ether anesthesia and blood was taken by heart puncture for serological studies. At autopsy, liver, spleen, kidneys and adrenals were removed, weighed, the former three frozen and stored on dry ice for subsequent enzymic activity determinations. Heart, excised portions of thigh musculature and one adrenal gland were fixed with 8 % formalin, sectioned and stained, while the other adrenal was used immediately for quantitative determination of its vitamin C content. The colorimetric micro-procedure of BESSEY and KING (10) was used for that purpose. The readings were performed in a Beckman quartz spectrophotometer model DU.

The degree of scurvy was appreciated by the presence and intensity of hemorrhages and by peculiar changes of the general habitus (11). In addition parts of the lower jaw, containing the roots of the incisor teeth, were decalcified, sectioned and used for histological estimation of the degree of scurvy present in the individual animals. Less specific signs of disease, such as slower increase or even loss of weight, could not be definitely ascribed to vitamin C deficiency, considering the complexity of the treatment applied.

The serological relation of the condition studied to human rheumatic disease was studied using the differential hemagglutination titers of the sera obtained against sensitized resp. unsensitized sheep erythrocytes. We are indepted to Dr. WICKMAN from the State Bacteriological Laboratory in Stockholm for generously performing those tests. Both the original technique of ROSE et al. (13) and its modification by BALL (14), yielding allegedly more clear-cut results with human rheumatic sera, were used for those determinations. The results of the above experiments and the references will be given in an accompanying publication (15).

Stockholm, University, Vitamin Institute.

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# Synthesis of (+)- and (−)-muscone

By Stina Ställberg-Stenhagen

In 1906 Walbaum (1) isolated from musc obtained from the musc-deer an optically active ketone, which he called »Muskon». This compound is responsible for the characteristic odour of natural musc. About twenty years later Ruzicka (2, 3) showed that the musc-smelling compounds of animal origin are macrocyclic ketones. From a study of the products obtained on oxidation of muscone with chromic acid, he arrived at the conclusion (4) that muscone was the laevorotatory enantiomorph of 3-methylcyclopentadecanone-1. DL-Muscone was first synthesized by Ziegler and Weber (5) in 1934, and the racemic compound has later been prepared by several different methods. Details of these may be found in the excellent review by Lederer (6). Although several of the cyclization methods described in the literature could be used for the synthesis of the optically active forms of 3-methylcyclopentadecanone-1, no synthesis of the laevorotatory, naturally occurring enantiomorph has been reported. This is no doubt due to the fact that the optically active forms of the β-methyl-substituted long chain dibasic acids required for the synthesis have previously been rather inaccessible. However, optically active acids of the type mentioned may be obtained in a straightforward manner from the enantiomorphs of methyl hydrogen β-methylglutarate (7) and a monoester of an appropriate normal chain dibasic acid with the aid of the Kolbe reaction (cf (8)).

The synthesis of the two enantiomorphs of 3-methylcyclopentadecanone-1 starting from the enantiomorphic 3-methylhexadecane-1,16-dioic acids will be described. The cyclization can be carried out by the method of Ziegler, Eberle and Ohlinger (9), or by the method recently described by Blomquist and Spencer (10). In the former case the dinitriles are required for the cyclization reaction. Ziegler's method gives excellent yields, but in the present case the method of Blomquist and Spencer was preferred, as it involves less labour.

The synthesis of the dextrorotatory enantiomorph of 3-methylcyclopentadecanone-1 is outlined in the chart. D(−)-Methyl hydrogen β-methylglutarate (I) when subjected to mixed electrolysis with methyl hydrogen tridecane-1,13-dioate (II) gave L (−)*-methyl 3-methylhexadecane-1,16-dioate* (IV) in a yield of 31 % of the theoretical. The ester (IV), which has an optical rotation $[\alpha]_D + 2.46°$ (homogeneous), was separated from the unavoidable by-products (III) and (V) by distillation. L(−)*-3-Methylhexadecane-1,16-dioic acid* (VI), $[\alpha]_D - 4.48°$ (chloroform; *c*, 10.1), $[M]_D - 13.4°$ was obtained by hydrolysis of the ester (IV). The dibasic acid has two crystalline forms, with melting points at 79.0–79.4° and 76.3–76.9° respectively.

Cyclization of the laevorotatory dibasic acid gave *dextrorotatory 3-methylcyclopentadecanone-1* (VIII) in a yield of 17 % of the theoretical. The optical rotation was $[\alpha]_D^{22} + 13.83°$ (homogeneous), $[M]_D^{22} + 33.0°$. The semicarbazone melted at 132.2–132.8°.

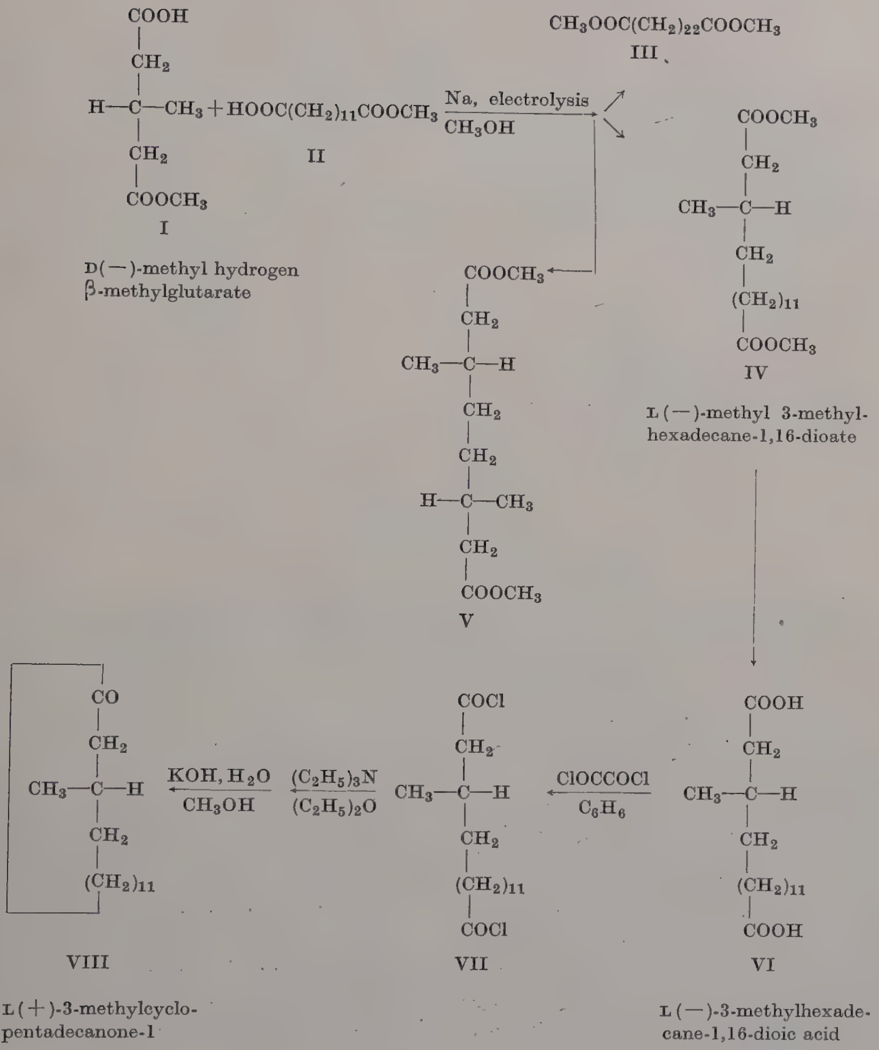
*Dextrorotatory 3-methylhexadecane-1,16-dioic acid* was prepared in analogous manner starting from L (+)-methyl hydrogen β-methylglutarate (for details see the experimental part), and cyclization of the corresponding acid chloride gave the naturally occurring laevorotatory muscone in a yield of 16 % of the theoretical. The optical rotation was $[\alpha]_D^{22} - 13.9°$ (homogeneous), $[M]_D^{22} - 33.1°$. The semicarbazone melted at 132.4–132.9°.

The optical rotation of muscone derived from musc is reported by RUZICKA (3) as $[\alpha]_D - 13.0°$. For the semicarbazone WALBAUM (1) gives m.p. 133–134°. RUZICKA (3) gives m.p. 134° for his best fraction, but later RUZICKA and STOLL (11) report a m.p. of 140–141°. It is pointed out by these authors, however, that high melting points of the semicarbazones may be due to the presence of small amounts of impurity. In the present case it was found that when equal amounts of the optically active semicarbazones were dissolved and the solvent evaporated, the resulting DL-semicarbazone melted about two degrees higher than the antipodes. The melting point now found for the DL-semicarbazone is 134.0–134.6°, which is in good agreement with the value given by ZIEGLER and WEBER (5), *viz.* 133.5–134.5°. HUNSDIECKER (12) gives m.p. 133–133.5°, RUZICKA and STOLL (11) 136–137°, and BLOMQUIST, HOLLEY and SPENCER (13) 131.5–132.5°.

The fact that laevorotatory muscone is obtained from dextrorotatory dibasic acid agrees with RUZICKA's finding (4) that the 3-methylpentadecane-1,15-dioic acid isolated from the products obtained by oxidation of natural muscone with chromic acid was dextrorotatory ($[\alpha]_D + 5°$). The 3-methyl-substituted dibasic acid just referred to results from oxidative cleavage between carbon atoms 1 and 15. The cleavage may also occur between carbon atoms 1 and 2, and in this case an optically active 2-methylpentadecane-1,15-dioic acid is produced. RUZICKA (3, 4) did not succeed in isolating this acid in a pure form, but he noted that the acid and its ester had a rotation of opposite sign compared with that of the 3-methyl-substituted acid. Optically active long chain 2- and 3-methyl-substituted monocarboxylic acids with the same configuration show optical rotations of different signs (14, 15). The active 3-methylhexadecane-1,16-dioic acids have the same molecular rotations as the active 3-methyl-substituted monocarboxylic acids of similar chain length (cf (16)), which indicates that the distant carboxyl group has a negligible effect on the optical rotation. The two dibasic acids derived from natural muscone have the same configuration, and they should therefore show optical rotations of opposite signs.

The steric relations between the optically active muscones and glyceraldehyde follow directly from the synthesis. When the formulae are written in the manner employed in the FISCHER convention (see chart) with carbon atom 1 (the carbonyl group) uppermost, the methyl group of natural muscone points to the right in the projection, and the systematic name is thus D (—)-3-methylcyclopentadecanone-1.

Chart.


COOH
CH2
H—C—CH3 + HOOC(CH2)11COOCH3
CH2
COOCH3
I
II
D(−)-methyl hydrogen β-methylglutarate
Na, electrolysis
CH3OH
CH3OOC(CH2)22COOCH3
III
COOCH3
CH2
CH3—C—H
CH2
(CH2)11
COOCH3
IV
L(−)-methyl 3-methylhexadecane-1,16-dioate
COOCH3
CH2
CH3—C—H
CH2
CH2
H—C—CH3
CH2
COOCH3
V
CO
CH2
CH3—C—H
CH2
(CH2)11
VIII
L(+)-3-methylcyclopentadecanone-1
KOH, H2O
CH3OH
(C2H5)3N
(C2H5)2O
COCl
CH2
CH3—C—H
CH2
(CH2)11
COCl
VII
ClOCCOCl
C6H6
COOH
CH2
CH3—C—H
CH2
(CH2)11
COOH
VI
L(−)-3-methylhexadecane-1,16-dioic acid

It is stated in the literature (11) that the smell of DL-muscone cannot be distinguished from that of natural laevorotatory muscone, and the author has not been able to detect any difference between the enantiomorphs with regard to their smell.

The rotatory dispersion has been measured for several of optically active compounds prepared. The data are given in Table I.

*Table I*

Molecular rotations at 25°.

| Compound | Wavelength Å | | | | | |
|---|---|---|---|---|---|---|
| | 3131 | 3650 | 4046 | 4358 | 5461 | 5893 |
| D(+)-Methyl 3-methylhexadecane-1,16-dioate (hom., *l*, 0.5) | | +27.6 | +20.5 | +16.9 | +9.8 | +8.1 |
| L(—)-Methyl 3-methylhexadecane-1,16-dioate (hom., *l*, 1 (micro tube)) | | —27.2 | —20.3 | —16.7 | —9.6 | —8.0 |
| D(+)-3-Methylhexadecane-1,16-dioic acid (chloroform; *l*, 2; *c*, 10) | +73 | +44.5 | +33.6 | +27.4 | +16.6 | +13.4 |
| L(—)-3-Methylhexadecane-1,16-dioic acid (chloroform; *l*, 2; *c*, 10) | —75 | —44.4 | —33.3 | —27.6 | —16.4 | —13.4 |
| D(—)-3-Methylcyclopentadecanone-1 (hom., *l*, 0.5 (micro tube)) | | | —105 | —79.9 | —40.5 | —32.9 |
| L(+)-3-Methylcyclopentadecanone-1 (hom., *l*, 0.2 (micro tube)) | | | +105 | +79.4 | +41.5 | +32.9 |

## Experimental[1]

### Methyl hydrogen tridecane-1,13-dioate

Tridecane-1,13-dioic acid was prepared from ethyl 11-bromohendecanoate by malonic ester synthesis. The dicarboxylic acid was converted into the dimethyl ester, b.p. 148°, 1 mm. The monomethyl ester was prepared from the dimethyl ester by Hunsdiecker's procedure (17).

Methyl tridecane-1,13-dioate (135 g) was dissolved in methanol (300 ml) and the calculated amount (435 ml) of 1.14 N barium hydroxide in methanol added. The mixture was kept at room temperature overnight. The precipitate was filtered off, ether being added to assist the filtration. The barium salt was decomposed by means of hydrochloric acid and the free monoester taken up in ether. The ether solution was washed with water and dried, the solvent distilled off, and the residue crystallized from light petroleum (b.p. 40–60°). The yield of methyl hydrogen tridecane-1,13-dioate was 111 g or 87 % of the theoretical. The monoester crystallized in the form of beautiful lustrous plates, which melted at 51.9–52.2°. After previous melting the m.p. was 52.0–52.2°. Noller and Adams (18), who prepared the monoester by oxidation of the corresponding ester aldehyde, give m.p. 57–57.5°. No definite explanation of this discrepancy can be given. It may be due to polymorphism.

Long X-ray spacing (pressed specimen) 33.6 Å.

### (—)-Methyl 3-methylhexadecane-1,16-dioate

D(—)-Methyl hydrogen β-methylglutarate (10 g, $\alpha_{\mathrm{D}}^{25.0}$—2.42° (homogeneous, *l*, 4), cf (7)), and methyl hydrogen tridecane-1,13-dioate (10 g) were added to a solution of sodium (0.4 g) in methanol (300 ml). The solution was subjected to electrolysis between platinum electrodes (cf (8)) in an externally water-cooled beaker of 400 ml capacity. Five platinum electrodes (two anodes) were used and a current of 8 amps was led through until the solution became neutral. Equal amounts (10 g) of the two half-esters were again added and the electrolysis continued until the solution was neutral. This was again repeated twice. The total time required for the electrolysis was 100 minutes. The mixture was allowed to cool. The crystalline precipitate was filtered off and washed on the filter several times with methanol. The filtrate was diluted with water and the mixture extracted with ether. The ether solution was washed with sodium carbonate solution, followed by water. The solution was dried (sodium sulphate), the ether distilled off, and the residue distilled *in vacuo*. The following fractions were collected.

---

[1] Meltings points, crystal spacings and optical rotations were determined in the manner described previously, cf *e.g.* (19) and (20).

| | | | | | |
|---|---|---|---|---|---|
| I | up to 82°, | 0.15 mm | 15 ml | | |
| II | 82–143°, | ” | 4 ” | | |
| III | 143–150°, | ” | 5.11 g | $\alpha_{\mathrm{D}}^{23}-2.21^\circ$ | (hom., $l$, 1) |
| IV | 150–152°, | ” | 5.61 ” | ” $-2.29^\circ$ | ( ” ) |
| V | 152°, | ” | 5.83 ” | ” $-2.29^\circ$ | ( ” ) |

The yield of (—)-methyl 3-methylhexadecane-1,16-dioate (fractions III, IV and V) was 31 % of the theoretical, calculated on the methyl hydrogen tridecane-1,13-dioate used.

$d_4^{25}$ 0.939

*Optical rotation* $\alpha_{\mathrm{D}}^{25}-2.31^\circ$ (homogeneous, $l$, 1)
$[\alpha]_{\mathrm{D}}^{25}-2.46$ $[\mathrm{M}]_{\mathrm{D}}^{25}-8.1^\circ$

*Anal.:* Calcd for $C_{19}H_{36}O_4$ (328.5) C, 69.47; H, 11.05 %
Found C, 69.11; H, 11.04 %

### (—)-3-Methylhexadecane-1,16-dioic acid

Hydrolysis of the above ester gave the corresponding dibasic acid, which after crystallization from benzene-light petroleum (b.p. 40–60°) (about 3:1 by volume) was obtained in microcrystalline form, m.p. 77.9–78.4°. After further crystallization from methanol the acid was macroscopically crystalline. The melting point was 79.0–79.4°. On very slow cooling solidification took place with the formation of small crystals which on reheating melted at the temperature just given. On more rapid cooling solidification occurred in the form of beautiful large plates which on heating melted at 76.5–76.9°. The dimorphism of the acid was confirmed by the X-ray diffraction patterns given by specimens crystallized from methanol and by specimens prepared by the hot-wire technique. The former (the high-melting form) gave a long spacing of 33.6 Å, while the long spacing of the latter was 29 Å.

*Optical rotation* $\alpha_{\mathrm{D}}^{24}-0.91^\circ$ (chloroform; $l$, 2; $c$, 10.1)
$[\alpha]_{\mathrm{D}}^{24}-4.48^\circ$ $[\mathrm{M}]_{\mathrm{D}}^{24}-13.4^\circ$

*Anal.:* Calcd for $C_{17}H_{32}O_4$ (300.4) C, 67.96; H, 10.73 %
Found C, 68.31; H, 10.71 %

### (+)-Methyl 3-methylhexadecane-1,16-dioate

The ester was prepared from L(+)-methyl hydrogen β-methylglutarate of $\alpha_D^{22} + 1.28°$ (homogeneous; *l*, 2) in the same manner as described for the antipode. The yield was 28 % of the theoretical.

*Optical rotation* $\alpha_D^{24} + 2.27°$ (homogeneous; *l*, 1)

*Anal.*: Calcd for $C_{19}H_{36}O_4$ (328.5) C, 69.47; H, 11.05 %
Found C, 69.48; H, 11.07 %

In order to get a check on the above value for the optical rotation, a specimen of the ester was prepared by means of diazomethane from the acid described below. B.p. 154.5–155.5°, 0.2 mm.

*Optical rotation* $\alpha_D^{25} + 1.16°$ (homogeneous; *l*, 0.5)
$[\alpha]_D^{25} + 2.47°$ $[M]_D^{25} + 8.1°$

### (+)-3-Methylhexadecane-1,16-dioic acid

The acid was obtained by hydrolysis of the ester obtained from the electrolysis. The acid showed the same behaviour as the antipode, the melting points of the two crystalline forms being 78.6–79.0° and 75.4–76.0° respectively. Long X-ray spacing (high-melting form) 33.8 Å.

*Optical rotation* $\alpha_D^{25} + 0.90°$ (chloroform; *l*, 2; *c*, 10.1)
$[\alpha]_D^{25} + 4.46°$ $[M]_D^{25} + 13.4°$

*Anal.*: Calcd for $C_{17}H_{32}O_4$ (300.4) C, 67.96; H, 10.73 %
Found C, 68.12; H, 10.80 %

### DL-3-Methylhexadecane-1,16-dioic acid

The racemic acid was prepared by crystallizing a mixture of equal amounts of the antipodes from methanol. The DL-acid was obtained in microcrystalline form, m.p. 76.8–77.4°. The acid solidified in the form of beautiful large plates, in a manner exactly similar to that of the low-melting form of the enantiomorphs. The solidified DL-acid remelted at 76.8–77.4°. The X-ray diffraction pattern of a pressed specimen appeared identical with that of the low-melting form of the antipodes.

Chuit *et al.* (21) give m.p. 77.2–77.4°, and Blomquist, Holley and Spencer (13) m.p. 74–76°.

### (+)-3-Methylcyclopentadecanone-1

The cyclization was carried out as described by Blomquist, Holley and Spencer (13). L(—)-3-Methylhexadecane-1,16-dioic acid (3.0 g) was converted into the acid chloride by means of oxalyl chloride (4.5 g) in benzene (10 ml) solution. The mixture was kept at a temperature of 40° for 3 hours. Excess oxalyl chloride and the solvent were distilled off under reduced pressure. In order to remove the oxalyl chloride completely, dry benzene (10 ml) was added and distilled off again. The acid chloride was dissolved in Grignard-dried ether

(250 ml), and the solution transferred to a HERSHBERG dropping funnel of 300 ml capacity.

The ether solution was added drop-wise over a period of 24 hours to a refluxing and stirred solution of triethylamine (15 ml) in Grignard-dried ether (600 ml), contained in a round-bottomed flask of 1000 ml capacity. About 500 ml ether were distilled off, and the remaining mixture was washed with dilute hydrochloric acid followed by water. The ether solution was dried with sodium sulphate, the ether distilled off, and the residue saponified by adding a solution of potassium hydroxide (3 g) in water (3 ml) and methanol (45 ml). The mixture was kept at room temperature for 24 hours and then at 40° for 36 hours. Water was added and the ketone extracted by means of ether. The ether solution was dried, the solvent distilled off, and the residue distilled in a micro-apparatus, giving 0.41 g (17 % of the theoretical) of (+)-*3-methylcyclopentadecanone-1* in the form of a colourless oil having the odour of musc. $d_4^{22}$ 0.918.

*Optical rotation* $\alpha_D^{22} + 6.35°$ (homogeneous; $l$, 0.5 (micro tube))
$[\alpha]_D^{22} + 13.83°$ $[M]_D^{22} + 33.0°$

*Anal.*: Calcd for $C_{16}H_{30}O$ (238.4) C, 80.60; H, 12.68 %
Found C, 80.81; H, 12.68 %

### (—)-3-Methylcyclopentadecanone-1

The laevorotatory enantiomorph was prepared from the dextrorotatory antipode of the dibasic acid. The procedure was that just described, except that the acid chloride in the dropping funnel was dissolved in thiophene-free benzene (dried over phosphorus pentoxide) instead of ether. 2.5 Grams of (+)-3-methylhexadecane-1,16-dioic acid gave 0.32 g (16 % of the theoretical) of laevorotatory muscone.

*Optical rotation* $\alpha_D^{22} — 2.55°$ (homogeneous; $l$, 0.2 (micro tube))
$[\alpha]_D^{22} — 13.9°$ $[M]_D^{22} — 33.1°$

*Anal.*: Calcd for $C_{16}H_{30}O$ (238.4) C, 80.60; H, 12.68 %
Found C, 80.51; H, 12.57 %

### Semicarbazone of (+)-muscone

The semicarbazone was prepared by the procedure used by BLOMQUIST, HOLLEY and SPENCER (13) for the DL-semicarbazone. After one crystallization from methanol the semicarbazone formed small plates melting at 129.5–130.5°. After two further crystallizations from methanol the m.p. was 132.2–132.8°.

Long X-ray spacing (pressed specimen) 17.1 Å.

*Anal.*: Calcd for $C_{17}H_{33}ON_3$ (295.4) N, 14.22 %
Found N, 14.39 %

### Semicarbazone of (—)-muscone

After one crystallization from methanol the semicarbazone melted at 129.5–130.5°. After one recrystallization from methanol the m.p. was 130.3–131.2°, and after two recrystallizations from the same solvent the semicarbazone formed small lustrous plates melting at 132.4–132.9°.

Long X-ray spacing (pressed specimen) 17.1 Å.

*Anal.:* Calcd for $C_{17}H_{33}ON_3$ (295.4) N, 14.22 %
Found N, 14.39 %

### Semicarbazone of DL-muscone

Equal amounts (25.4 mgs) of once crystallized antipodes (m.p. 129.5–130.5°) were dissolved in ether. The ether was allowed to evaporate, and the DL-semicarbazone thus obtained melted, without recrystallization, at 132.5–133.5°. After two crystallizations from methanol the DL-semicarbazone melted at 134.0–134.6°.

The X-ray diffraction pattern was indistinguishable from those of the enantiomorphs.

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### SUMMARY

The synthesis of the naturally occurring laevorotatory muscone and its antipode is described.

D(+)-3-Methylhexadecane-1,16-dioic acid is prepared via the methyl ester obtained by mixed electrolysis of L(+)-methyl hydrogen β-methylglutarate and methyl hydrogen tridecane-1,13-dioate in methanol solution. The dextrorotatory dibasic acid is converted into D(—)-3-methylcyclopentadecanone-1 (laevorotatory muscone) by the cyclization method of BLOMQUIST and SPENCER. The optical rotation of the synthetic muscone is slightly higher than that reported for the natural product.

L(+)-3-Methylcyclopentadecanone-1 is prepared in an analogous manner starting from D(—)-methyl hydrogen β-methylglutarate.

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REFERENCES. 1. **Walbaum, H.**, J. prakt. Chem. (2) *73*, 488 (1906). — 2. **Ruzicka, L.**, Helv. Chim. Acta *9*, 230 (1926). — 3. *Idem, ibid. 9*, 715 (1926). — 4. *Idem, ibid. 9*, 1008 (1926). — 5. **Ziegler, K.**, and **Weber, K.**, Annalen *512*, 164 (1934). — 6. **Lederer, E.**, "Odeurs et parfums des animaux", Progress in the chemistry of organic natural products, Vol. 6, p. 87, Vienna 1950. — 7. **Ställberg-Stenhagen, S.**, Arkiv för Kemi, Min. o. Geol. *25 A*, N:o 10 (1947). — 8. *Idem*, Arkiv för Kemi *2*, 95 (1950). — 9. **Ziegler, K.**, **Eberle, H.**, and **Ohlinger, H.**, Annalen *504*, 94 (1933). — 10. **Blomquist, A. T.**, and **Spencer, R. D.**, J. Amer. Chem. Soc. *70*, 30 (1948). — 11. **Ruzicka, L.**, and **Stoll, M.**, Helv. Chim. Acta *17*, 1308 (1934). — 12. **Hunsdiecker, H.**, Berichte *75*, 1197 (1942). — 13. **Blomquist, A. T.**, **Holley, R. W.**, and **Spencer, R. D.**, J. Amer. Chem. Soc. *70*, 34 (1948). — 14. **Levene, P. A.**, and **Marker, R. E.**, J. Biol. Chem. *98*, 1 (1932). — 15. **Ställberg-Stenhagen, S.**, Arkiv för Kemi *1*, 153 (1949). — 16. *Idem, ibid. 1*, 187 (1949). — 17. **Hunsdiecker, H.**, and **Hunsdiecker, Cl.**, Berichte *75*, 291 (1942). — 18. **Noller, C. R.**, and **Adams, R.**, J. Amer. Chem. Soc., *48*, 1074 (1926). — 19. **Ställberg-Stenhagen, S.**, Arkiv för Kemi, Min. o. Geol. *23 A*, N:o 15 (1946). — 20. **Ställberg-Stenhagen, S.**, and **Stenhagen, E.**, J. Biol. Chem. *173*, 383 (1948). — 21. **Chuit, P.**, **Boelsing, F.**, **Hausser, J.**, and **Malet, G.**, Helv. Chim. Acta *10*, 167 (1927).

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# Nuclear chemical investigation of the alcohol reduction of diazonium salts

By LARS MELANDER

In connexion with another investigation it was tried to introduce tritium into the aromatic nucleus by reducing a diazonium salt with ethanol containing tracer amounts of tritium in the hydroxy group (1 a). The reaction, the gross formula of which is

$$\text{ArylN}_2^+ + C_2H_5OH \rightarrow \text{ArylH} + N_2 + CH_3CHO + H^+$$

has sometimes been written, at least schematically, in such a manner that the hydroxy hydrogen could be supposed to enter the aromatic position. The negative result of the attempt referred to showed that this could hardly be the case.

The experiments to be described in the present paper were undertaken in order to show that it is one of the methylene hydrogens that enters the aromatic molecule, and simultaneously to measure the relative reaction rates of tritium and protium. Data of this type will be useful in the discussion of the reaction mechanism. The investigation was possible owing to the fact that the hydrogens attached to carbon in the alcohol molecule do not take part to any appreciable extent in the general interchange of hydrogen nuclei between the hydroxy group, the water, the sulphuric acid present in the system, etc.

ALEXANDER and BURGE (2) have studied the behaviour of deuterium in the analogous reduction of m-nitrobenzenediazonium ion with aqueous hypophosphorous acid. Owing to some hydrogen exchange reactions, the rates of which are unknown, it was not possible to measure the isotopic effect on reaction velocity very accurately. The results, which seem to be consistent with those of the present investigation, will be discussed below.

The reduction of a diazonium ion represents a favourable case for the measurement of the relative reaction rates of hydrogen isotopes, even down to tracer amounts, for it is possible to catch and purify the product containing the transferred hydrogen. Generally in similar reactions involving a reduction (oxidation of the alcohol) in aqueous systems water is formed from the hydrogen, which will then intermix with hydrogens of other origin.

Thus in the deuterium experiments on the mechanism of the chromic acid oxidation of propanol-2 carried out by WESTHEIMER and NICOLAIDES (3), the relative rates of attack on propanol-2 and 2-deuteropropanol-2 were calculated from the rates with which aqueous chromic acid was consumed by different mixtures of the two isotopic alcohols. Such measurements are not very sensitive to isotopic effects on the reaction velocity unless preparations of high isotopic purity in the reactive position are available or the change in isotopic ratio of the remaining initial compound at known and not too low yields is studied.

The results of WESTHEIMER and NICOLAIDES, who found the removal of the secondary hydrogen of the propanol to be rate-controlling, are related to those of the present investigation, in which the diazonium ion acts as an oxidant, and they will be discussed below.

## Outlines of the experiments

Tracer amounts of tritium, originally present as water, were used. In the experiments with laballed hydrogen at the oxygen atom no particular synthesis of the alcohols was required since these hydrogens exchange immediately with water. As water occurs in the reaction mixture anyhow, addition of the radioactive water will be sufficient. It may be assumed that the tritium will distribute itself almost uniformly among the oxygens of the water and those of the alcohol, for the bonds are of similar type. Generally the water was added to the alcohol from the beginning, i.e. before the diazotization. Only in the first experiment it was added just before the beginning of the reduction (1 a).

Ethanol-1-t was prepared by catalytic hydrogenation of acetaldehyde with hydrogen prepared from the radioactive water. The tritium at the oxygen was removed by means of exchange with ethylene glycol, which is more easily separated from the ethanol than water is. However, the nickel catalyst used might be suspected to cause hydrogen exchange even in the methyl group. This would do little harm if only the distribution of tritium between the methyl and methylene groups could be accurately determined. Therefore a sample of the ethanol was oxidized to acetic acid with dichromate in dilute sulphuric acid, and the methylene hydrogens thus eliminated. The acetate ion was precipitated as sodium uranyl acetate, and the tritium determined. Check experiments, however, revealed a slight hydrogen exchange with the water in this process, probably due to the intermediate acetaldehyde. When these experiments were carried out, the work of CORNFORTH and POPJÁK (4) had not yet been observed. They claim that the oxidation with aqueous potassium permanganate proceeds without such an exchange, and that method might be preferable. Here only a lower limit of the tritium content of the methyl group was obtained, corresponding to an upper limit for the methylene group, probably not very far from the true value.

Also the method of CORNFORTH and POPJÁK (4) for the preparation of methylene-labelled ethanol might have been preferable, as no deuterium appeared in the methyl group. No attempt was made to prepare ethanol-1-t by reducing a suitable compound with lithium aluminium hydride, partly owing to the lack at that time of a simple method for the preparation of the corresponding triterated compound in good yield. Such a method has recently been published (5), but still the necessity of controlling the position of the tritium in the final ethanol can hardly be denied, for hydrogen exchange during the hydrogenation does not seem quite improbable.

In order to enable analogous experiments to be made without that uncertainty, methanol-t with tritium at the carbon was prepared. After some exchange and hydrogenation experiments the best method of preparation was found to be the methylation of benzoic acid containing tritium in the acid position with diazomethane. If a small amount of water containing the tritium is mixed with a large amount of benzoic acid in ether solution the movable hydrogens will interchange, and at equilibrium most of the tritium will be present as $C_6H_5COOT$. If the hydrogen mass has no strong influence on the reaction rate, a good yield of $C_6H_5COOCH_2T$ might be expected if the mixture is treated with an excess of diazomethane. The ester is easily separated and purified and will on saponification give a methanol of high purity. The ease with which methanol is separated from water by means of distillation is another advantage of using it instead of ethanol.

The ortho and meta isomers of nitrobenzenediazonium ion, which undergo alcohol reduction with reasonable yields, were used. The diazotization of the corresponding nitroanilines as sulphates was carried out in the triterated alcohols themselves, and a short while after the addition of the sodium nitrite was complete, the mixture was heated and the reduction thus started. For the reactions the description given by CLARKE and TAYLOR (6) for the preparation of m-nitrotoluene out of 3-nitro-4-aminotoluene was followed in principle. The alcohol was always present in a large excess. In the cases of the meta isomer the solubility was insufficient and the diazotization was carried out on a suspension. The low yields with that isomer may at least partly be explained by ineffective stirring.

The temperature at which the reduction starts is not known sharply, but measurements of the temperature of the mixture show that the evolution of nitrogen does not set in much below the boiling point of the mixture, at least in the case of methanol. If the reduction follows the splitting off of nitrogen, which is not certain (see below), the temperature will be about 60° C. If a more elaborate discussion of the magnitude of the isotope effect is to be carried through, experiments enabling a better temperature controll should be arranged.

After the reduction and before the working up of the mixture about half of the alcohol was distilled off. This alcohol sample may be investigated as to possible exchange during the reactions. The tritium in the hydroxy group is easily removed by exchange with water (for methanol) or ethylene glycol (for ethanol). The remaining tritium content should be the same as the original one in the case of tritium attached to carbon and otherwise zero.

The final nitrobenzene, which is known to be fairly insusceptible to electrophilic attacks, will certainly not exchange its hydrogen with the surroundings.

Methanol as the reducing agent is known to give fair amounts of the methoxy compounds corresponding to the initial diazonium ions (7), and even with ethanol the ethoxy compounds are common as by-products. As tritium enters the benzene ring much slower than does protium, and the same will certainly not apply to the alkoxy-t groups as compared to the ordinary ones, the presence of even slight amounts of the ethers will cause an erroneously high tritium content in the final preparation of nitrobenzene. The same may hold for impurities of other kinds. In order to transform the problem into one of purification of a solid substance, which might be purified by means of crystallization, the nitrobenzene was converted into m-dinitrobenzene and the latter recrystallized from ethanol. In the case of nitrobenzene-3-t, which is formed from the m-nitrobenzenediazonium ion, the tritium is affected in the nitration, but it is known (1) that the rate of nitration is equal for the two isotopes, and that no exchange occurs in the reaction. Thus equal amounts of m-dinitrobenzene-5-t and ordinary m-dinitrobenzene are formed from nitrobenzene-3-t, and half the tritium content is lost. Calculations should be based upon the measurements of the tritium content of the m-dinitrobenzene, and in all cases the nitrobenzene was found to have a proportionally higher tritium content. This fact is possible to explain only on the assumption of tritium-rich impurities.

## Experimental[1]

All melting and boiling points given in the following are uncorrected.

*Preparation of tritium.* The tritium was obtained from beryllium bombarded with 6.5 MeV deuterons in the small cyclotron of the institute. The simple method of preparing tritium-containing water from the tritium trapped in the beryllium filings has been described in a previous paper (8). The specific activity of the water used for the preparation of alcohols was of the order of magnitude of $10^7$ disintegrations per minute per millimole.

*Determination of tritium.* The method of determining tritium, founded on the conversion of the water to hydrogen and "inside counting" of the latter, has also been described previously (8).

The organic molecules were burned, and the water formed used for tritium determinations as above. The alcohols were burned in oxygen over the ordinary Reihlen and Weinbrenner combustion tube filling. The sodium uranyl acetate was contained in a copper boat and covered with powdered anhydrous potassium dichromate; the combustion layer and the atmosphere were the same as for the alcohols. The substances containing nitrogen, finally, were burned over cupric oxide in a stream of carbon dioxide, and the oxides of nitrogen reduced with reduced and heated copper.

The sources of error in the different processes have been discussed in a more recent paper (1 b) and empirical material has been presented. It may be concluded that the error in the determination of a specific tritium activity of intermediate strength (as for the alcohols) seldom exceeds ± 2 %. In the present investigation, however, several rather weak specific activities (most of the reaction products) have been determined, and the estimated error is then larger.

*Preparation of ethanol-1-t (together with some ethanol-2-t).* Freshly distilled acetaldehyde (Eastman Kodak Co., "for chemical purposes") was hydrogenated at atmospheric pressure and about 170° C with tritium-containing hydrogen over a catalyst of nickel supported on kieselguhr (9). Part of the hydrogen was prepared from water (about 100 mg) containing tritium by means of reduction with magnesium turnings at about 575° C in a stream of ordinary hydrogen. With proper adjustment of the rate of flow through the catalytic furnace and of the ratio between acetaldehyde and hydrogen it was possible to get roughly three fourths of the hydrogen consumed and about 60 % of the tritium introduced in the resulting ethanol.

In the following purification steps 99.5 % ethanol was added successively in order to wash out most of the activity and decrease the activity losses. The original volume of the sample was of the order of 1 ml, and when it was to be used a volume of about 60 ml was wanted.

The ethanol was distilled in a small column of the Podbielniak type. The fraction below 77.0° C was wasted (acetaldehyde) and the fraction 77.0—77.9° C collected. The latter was mixed with about 10 times the volume of freshly distilled ethylene glycol. The most volatile part, up to 194° C, was distilled off (no column was used) and added to a similar portion of ethylene glycol. The fraction up to 194° C was again distilled off and transferred to the small Podbielniak column. The fraction 77.4—78.6° C was collected and used for the experiments after further dilution.

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[1] Ing. K. Halvarson has carried out a good deal of the preparative work, and the writer takes this opportunity to thank him.

Samples of the two glycol portions were purified by means of distillation and their tritium content measured. Just as expected the first one contained more than ten times as much tritium as the second. The final yield of ethanol-1-t together with ethanol-2-t was 30 % as calculated on the original tritium. On the assumption of a uniform tritium distribution over all hydroxy groups it can be calculated from the specific activity of the last glycol portion that less than 0.3 % of the tritium in the final ethanol was situated at the oxygen.

*Attempt to determine ethanol-2-t.* 1.5 ml of the ethanol containing ethanol-1-t and ethanol-2-t was oxidized with sodium dichromate in dilute sulphuric acid according to MANN and SAUNDERS (10). Standard sodium hydroxide was added to the acetic acid in the distillate to make phenolphthalein red (13.2 mmol). The solution was evaporated practically to dryness in order to expel acetaldehyde etc. The residue was dissolved in 5 ml of water and the solution made just neutral with a few drops of weak hydrochloric acid. The solution was heated and added to a heated solution of 2.2 g of uranyl nitrate (with 6 molecules of water of crystallization) in 5 ml of water. The sodium uranyl acetate crystallized immediately, and the last sodium acetate was washed down into the mixture with 2 ml of water. After a short heating the mixture was allowed to cool in a refrigerator. The crystals were filtered off, washed with a small amount of cold water, and dried at 148° C. After a while the crystals were powdered and dried again for several hours. A gravimetric determination of hydrogen (the compound was burned as in the determination of tritium) gave 1.92 % H, calculated 1.93 %.

The tritium contents of the ethanol and the sodium uranyl acetate showed that 11.3 % of the tritium in the former was still present in the acetate ion, i.e. originated from the methyl group. Exchange during the reaction would have decreased the figure, and it could be stated that at least 11.3 % of the activity was due to ethanol-2-t.

When the exchange of hydrogen during the reactions just described was to be investigated, ordinary 99.5 % ethanol was oxidized and finally converted into sodium uranyl acetate according to the same method but with some radioactive water added to the aqueous phases, both at the oxidation and at the precipitation. The only difference in the treatment was that the crystals were not washed after they had been filtered off. From the specific activity of the radioactive water it was calculated that the mean specific activity of all hydrogens in the system was never below 47.5 units.[1] The hydrogen obtained from the sodium uranyl acetate had the specific activity 2.88 or 0.06 of the mean. Prolonged drying of the compound did not change the value appreciably.

*Preparation of $CH_2TOH$.* 120—150 mg of radioactive water and 4—5 g of benzoic acid (Coleman & Bell Co., c.p.) were dissolved in 160—200 ml of dry ethyl ether. The solution was placed in an ice bath and diazomethane gas carried by nitrogen was introduced. The diazomethane was prepared from hydrazine and chloroform (11), and when only 4 g of benzoic acid was used one batch given in the description referred to was sufficient. The excess of diazomethane was removed by addition of solid benzoic acid till the solution turned colourless. A slight amount of a white precipitate was formed in the reaction. It was filtered off. The ether was evaporated, and the ester was distilled at 10—20 mm mercury pressure within an interval of about 5°.

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[1] The unit of specific activity refers to the particular Geiger-Müller tube used throughout this investigation and corresponds to about 250 disintegrations per millimole hydrogen per minute.

The purified methyl benzoate (3.5—5 g) together with 30—40 ml of 10 % sodium hydroxide and preferably also 5 ml of pure methanol (pro analysi, LKB-Produkter) was boiled under reflux for one hour. After the solution had cooled down, the condenser was washed with methanol, and the mixture, which now contained 10—15 ml of extra methanol, was distilled through a Widmer column. The distillate up to about 70° C was collected, some more methanol added to the residue, and the distillation continued. The joined distillates were distilled again in the same apparatus after the aqueous residue had been removed. When the distillation bulb was almost empty, inactive methanol was added and distilled off. The same "washing out" of the activity was repeated once more. The result was about 33 ml of methanol, which contained 51—52 % of the initial tritium and all parts of which boiled within 1°.

In experiment 7 (see below) methanol distilled off in the other experiments was used after having been furthermore distilled within an interval of 1.7°.

*Diazotization and reduction.* In the main the description given by CLARKE and TAYLOR (6) for the preparation of m-nitrotoluene from 3-nitro-4-aminotoluene was followed. For experiment 5 a m-nitroaniline prepared from m-dinitrobenzene (Kebo) according to Orthner-Reichel (12), m.p. 112—114° C, was used. In all other experiments o- or m-nitroaniline "for chemical purposes" from Eastman Kodak Co. was used.

In the experiments with tritium in the hydroxy group, except No. 1 which has been described in a previous paper (1 a), about 60 mg of radioactive water was added to 30 ml of the alcohol before the diazotization.

To 30 ml of alcohol (in experiment 5 27.5 ml of ethanol plus 1.1 ml of ordinary water) was added 6.9 g (0.05 mol) of nitroaniline and with simultaneous cooling in an ice bath 6.2 ml of concentrated sulphuric acid. With constant stirring and the temperature being kept below 10° C by means of an ice bath, a solution of 3.9 g of sodium nitrite in 5.5 ml of water was added drop by drop. After the addition had been completed, the solution was stirred further 10 minutes.

The mixture was heated gently on a water bath under a reflux condenser and subsequently boiled for one hour (in experiment 5 it was only heated until the evolution of gas had ceased). 13—14 ml of the alcohol was then distilled off through a column, generally one of the Widmer type. After the distillation the residue was diluted with 150 ml of water and the nitrobenzene distilled off with steam. The nitrobenzene was separated and the aqueous phase of the distillate extracted with about 10 ml of benzene. The benzene was distilled off at reduced pressure from the liquids, which had been combined and dried with calcium chloride, and finally the nitrobenzene was distilled at 10—20 mm, generally within 2°. The final yield varied between 8 and 62 % of the theoretical amount. The refractive index was controlled.

*Investigation of alcohols distilled off.* In some experiments the alcohol, which had been distilled off, was investigated. In the case of ethanol (13.5 ml) it was mixed with 100 ml of freshly distilled ethylene glycol and the mixture distilled. The first 2 ml were wasted, and the fraction 110—128° C (no column was used) collected (8 ml). The distillate was again mixed with a new 50 ml portion of glycol, distilled, and the main fraction (4 ml, 100—130° C) collected. These 4 ml were finally distilled in the small Podbielniak column, and 1.1 ml of the most constant boiling fraction (76.5—77.0° C) collected.

In the case of methanol essentially the same method was followed but with water (5 times the volume of the alcohol) instead of glycol, and with the Widmer distillation column. After two distillations with water, the resulting methanol was distilled without water in the Podbielniak column, and an intermediate fraction (within 0.7°) was collected.

*Nitration of nitrobenzene.* The following procedure was used in the nitration of nitrobenzene, sometimes on a somewhat smaller scale owing to the scarcity of nitrobenzene. 0.5 ml of nitrobenzene was shaken in a closed vessel for 4 minutes with 8 ml of a mixture of 1 part by volume of concentrated nitric acid (sp.gr. 1.40) and 2 parts of concentrated sulphuric acid (sp.gr. 1.84). After the shaking the mixture was poured into ice water. The solid was filtered off, washed with water, and recrystallized from ethanol. The m-dinitrobenzene was finally dried over calcium chloride under reduced pressure. The melting point was controlled.

## Calculations

In the reduction the alcohols are present in an amount at least ten times the theoretical one. Thus the relative concentrations of the two isotopic species will not change much even though they are consumed with different rates. However, as it is possible to calculate the rate ratio rather accurately, the influence of the slight concentration change on the result should be investigated.

In the following calculations side reactions of the alcohol should be taken into account. For instance the formation of aliphatic-aromatic ethers is probably extensive in some cases. If it is assumed that the rate of such reactions is not influenced by differences in the isotopic mass of one hydrogen at carbon, the side reactions could be accounted for in a simple manner.

Let $AH_n$ represent a molecule of alcohol with $n$ hydrogen atoms in the reactive position, and $RH$ the final deamination product. More than one tritium atom will never occur in the same molecule, and the following reaction possibilities exist for the alcohol molecules:

$$AH_n \begin{cases} \xrightarrow{\quad n k_H \quad} RH \\ \xrightarrow{\quad q \quad} \text{by-products} \end{cases}$$

$$AH_{n-1}T \begin{cases} \xrightarrow{(n-1)\, k_H} RH \\ \xrightarrow{\quad k_T \quad} RT \\ \xrightarrow{\quad q \quad} \text{by-products} \end{cases}$$

where the specific rates are given above the arrows ($q$ accounts for all side reactions). $k_H$ and $k_T$ are the specific rates for the reaction of one specified hydrogen atom. It seems legitimate to assume that the reactions are of the first order with respect to the alcohol, and then if the concentrations of $AH_n$ and $AH_{n-1}T$ are represented by respectively $i$ and $a$ (initial values $i_0$ and $a_0$) the following equation holds:

$$\frac{-da}{-di} = \frac{(n-1)k_H + k_T + q}{n k_H + q} \cdot \frac{a}{i} = \frac{n-1+\beta+\varepsilon}{n+\varepsilon} \cdot \frac{a}{i}$$

where $\beta = k_T/k_H$ and $\varepsilon = q/k_H$. Integration gives

$$\frac{a}{a_0} = \left(\frac{i}{i_0}\right)^{\frac{n-1+\beta+\varepsilon}{n+\varepsilon}}$$

The fraction of radioactive alcohol in the total alcohol is very close to the ratio between radioactive and ordinary alcohol. The initial value of this ratio is

$$y_0 = a_0 / i_0$$

The corresponding ratio for the product $RH$ will be

$$u = \frac{\dfrac{k_T}{(n-1)k_H + k_T + q}(a_0 - a)}{\dfrac{n k_H}{n k_H + q}(i_0 - i)} = \frac{\beta(n+\varepsilon)}{n(n-1+\beta+\varepsilon)} \cdot \frac{a_0 - a}{i_0 - i}$$

The fraction of the ordinary alcohol that has been consumed is denoted by $x = \frac{i_0 - i}{i_0}$. The following expression may be derived from the equations above:

$$\frac{u}{y_0} = \frac{\beta(n+\varepsilon)}{n(n-1+\beta+\varepsilon)} \cdot \frac{1}{x}\left[1 - (1-x)^{\frac{n-1+\beta+\varepsilon}{n+\varepsilon}}\right]$$

For small values of $x$ the following expansion is useful:

$$\frac{u}{y_0} = \frac{\beta}{n}\left[1 + \frac{1-\beta}{2(n+\varepsilon)}x + \cdots\right]$$

and $\beta$ could be calculated approximately

$$\beta = n\frac{u}{y_0}\left[1 - \frac{1-\beta}{2(n+\varepsilon)}x\right]$$

What is measured, however, is the average specific activity of the total hydrogen of a compound. If the alcohol molecule contains in all $N_A$ hydrogens, and the final molecule $N_R$ hydrogens, and $z_0$ and $v$ are the respective specific activities,

$$\frac{u}{y_0} = \frac{N_R}{N_A} \cdot \frac{v}{z_0}$$

and $\beta$ is calculated from

$$\beta = n \frac{N_R}{N_A} \cdot \frac{v}{z_0} \left[ 1 - \frac{1 - \beta}{2(n + \varepsilon)} x \right]$$

The last formula may be used even when a derivative of $RH$ is measured if only $N_R$ is modified correspondingly and a correction is applied for activity loss if any occurs, as in the nitration of nitrobenzene-3-t.

The theoretical yield has hardly been obtained in any of the experiments, even if the measured yield may be considerably lower than the true one owing to losses during the working up of the reaction mixture. However, $x$ is the fraction of alcohol used up in all reactions together. It seems reasonable to assign a value to $x$ corresponding to the consumption of one molecule of alcohol for each molecule of nitroaniline used, and this value is called $x_{\max}$.

In experiment 5 the amount of ethanol was only 27.5 ml, corresponding to $x_{\max} = 0.106$. The lowest possible $\beta$ is roughly 0.1. The largest value of the correction is obtained if $\varepsilon = 0$, thus

$$- \frac{1 - \beta}{2(n + \varepsilon)} x_{\max} = -0.024$$

or —2.4 % is the highest value needed for the correction. The estimated error in $\beta$ is more than 10 % in the ethanol experiments, and so the correction may be left out, especially as the neglect counteracts the probably positive error in the value of $z_0$ for ethanol-1-t.

In the methanol experiments the corresponding values are $x_{\max} = 0.068$, $\beta = 0.14$, and $\varepsilon = 0$, which gives a correction of —1.0 %. The estimated error is in no case less than 5 %, and the correction may be neglected.

Thus the value of $\beta$ may be calculated as if the isotopic composition of the alcohol maintained its original value throughout the reaction, and this is justified also by the result of the experiment, in which $CH_2TOH$ was recovered and investigated after it had taken part in one of the reactions. The formula according to which $\beta$ may be calculated is then simply

$$\beta = n \frac{N_R}{N_A} \cdot \frac{v}{z_0}$$

## Results

In the experiments with tritium in the hydroxy group of the alcohol probably no tritium at all was introduced into the aromatic compound by reduction. In Table I the results, however, have been given as the upper limit of $\beta = k_T / k_H$ in such a hypothetical reaction. $\beta_1$ and $\beta_2$ are calculated from the specific activities $v_1$ and $v_2$ of respectively nitrobenzene and dinitrobenzene. $z_0$ has been calculated as the specific activity of the movable hydrogen of the system assuming a uniform distribution of tritium between all hydrogens of the water and the sulphuric acid, two hydrogens originating from the amino group of the nitroaniline, and one of the alcohol. Since $z_0$ refers only to the hydroxy hydrogen of the alcohols, $N_A$ should be assigned the value 1 in the formula for the calculation of $\beta$. $v$ and $z_0$ have been corrected to the same point of time, assuming 12.0 years as the half-life of tritium. For safety $\beta_2$ in experiment 3 has been calculated on the assumption of a loss of half the amount of tritium in the nitration. Experiment 1 has been reported previously (1 a).

*Table I.*

Reduction of diazonium ions with alcohols containing tritium in the hydroxy group. For symbols, cf. the text, for unit of $z_0$, $v_1$, and $v_2$, foot-note 1 on page 529.

| Exp. | Alcohol | Diazonium ion | $z_0$ | $v_1$ | $v_2$ | $\beta_1$ | $\beta_2$ |
|---|---|---|---|---|---|---|---|
| 1 | Ethanol | m-nitrobenzene- | 52 | $\leq 0.22$ | | $\leq 0.021$ | |
| 2 | Methanol | o-nitrobenzene- | 116 | $< 0.1$ | $< 0.1$ | $< 0.0043$ | $< 0.0035$ |
| 3 | Methanol | m-nitrobenzene- | 110 | 0.74 | $< 0.2$ | 0.034 | $< 0.015$ |

Ethanol-1-t was always contaminated with some ethanol-2-t. The two samples used had the same origin and the approximate determination of the percentage of ethanol-2-t described in the experimental part applies to both. The value of $z_0$ in Table II is accordingly the upper limit of the specific activity due to ethanol-1-t. The calculated $\beta$ will correspondingly represent a lower limit, but the estimated limits of error are supposed to cover the true value. In the column of $v_2$ the specific activities of two different preparations of *m*-dinitrobenzene and their mean value with its estimated error have been given. Only $\beta_2$, being the most reliable value, has been given. In the sixth column the tritium contents of nitrobenzene and dinitrobenzene have been compared. If both had been pure, the result should have been unity. In the calculations of experiment 5 the loss of tritium in the nitration has been accounted for, and corrections for decay have been applied. The error in $z_0$ is estimated at 2 % (1 b).

*Table II.*

Reduction of diazonium ions with ethanol-1-t.
For symbols, cf. the text, for unit of $z_0$, $v_1$, and $v_2$, foot-note 1 on page 529.

| Exp. | Diazonium ion | $z_0$ | $v_1$ | $v_2$ | Rel. tritium content | $\beta_2$ |
|---|---|---|---|---|---|---|
| 4 | o-nitrobenzene- | 14.4 | 1.88 | $\left.\begin{matrix}1.99\\2.13\end{matrix}\right\}2.06 \pm 0.10$ | $\frac{5\,v_1}{4\,v_2} = 1.14$ | $0.19 \pm 0.02$ |
| 5 | m-nitrobenzene- | 17.3 | 1.57 | $\left.\begin{matrix}0.83\\0.88\end{matrix}\right\}0.86 \pm 0.10$ | $\frac{5\,v_1}{2{\cdot}4\,v_2} = 1.14$ | $0.13 \pm 0.02$ |

Table III shows the corresponding results obtained with methanol-1-t.

The results from the investigation of samples of the alcohols used in the experiments 3, 4, and 8 have been collected in Table IV. The initial specific activity of the methanol in experiment 3 is given as one fourth of the value of $z_0$ in Table I. If the tritium had been uniformly distributed among the movable hydrogen positions, the alcohol separated from the mixture and its tritium determined, this value would have been obtained. The decay of tritium has been accounted for.

*Table III.*

Reduction of diazonium ions with methanol-1-t.
For symbols, cf. the text, for unit of $z_0$, $v_1$, and $v_2$, foot-note 1 on page 529.

| Exp. | Diazonium ion | $z_0$ | $v_1$ | $v_2$ | Rel. tritium content | $\beta_2$ |
|---|---|---|---|---|---|---|
| 6 | o-nitrobenzene- | 58.0 | 2.38 | $2.84 \pm 0.08$ | $\frac{5 v_1}{4 v_2} = 1.05$ | $0.147 \pm 0.008$ |
| 7 | o-nitrobenzene- | 38.4 | 1.60 | $1.92 \pm 0.08$ | $\frac{5 v_1}{4 v_2} = 1.04$ | $0.150 \pm 0.009$ |
| 8 | m-nitrobenzene- | 65.5 | 6.53 | $1.56 \pm 0.07$ | $\frac{5 v_1}{2{\cdot}4 v_2} = 2.62$ | $0.143 \pm 0.010$ |

*Table IV.*

Investigation of alcohols used in reduction experiments.
For unit of specific activity, cf. foot-note 1 on page 529.

| Exp. | Alcohol | Specific activity of alcohol | |
|---|---|---|---|
| | | initial | final, after removal of hydroxy tritium |
| 3 | $CH_3OT$ | 27.5 | < 0.15 |
| 4 | $CH_3CHTOH$<br>$CH_2TCH_2OH$ | 16.2 | 15.1 |
| 8 | $CH_2TOH$ | 65.5 | 66.2 |

## Discussion of results

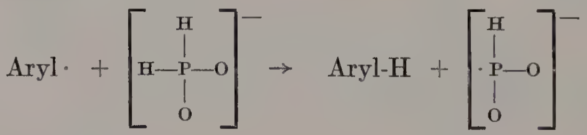
The results given in Table IV show that the hydrogen atoms attached to carbon in the alcohols do not interchange with the movable hydrogen of the system. The specific activity of the recovered ethanol from experiment 4 is a little low, but the difficulty of separating ethanol from water should be kept in mind. In the final steps it was distilled from freshly distilled ethylene glycol, but no other special measures to make it anhydrous were taken.

According to Table I probably no hydrogen from the hydroxy group is introduced into the aromatic ring, for isotopic effects of the magnitude 1: 100 are hardly possible under the conditions in question. If a more probable effect of 1: 10 is assumed, only about one tenth or less of the molecules could be supposed to react along this path. In fact, there is only one case in which a real tritium content has been found in a product, in all others the true value may be zero.

The only product having a measurable activity was the nitrobenzene from m-nitrobenzenediazonium ion and methanol in experiment 3. Also in experiment 8, in which the tritium was located at the carbon of the methanol, a similar anomalously high tritium content was observed in the nitrobenzene as compared to the m-dinitrobenzene. Just in these two experiments the crude nitrobenzene was particularly impure, as was noticed in the final distillation. It is remarkable that the formation of a methoxy compound cannot account for the result in experiment 3, for the methyl should be and has also been shown to be free of tritium. The fact that most, if not all, of the tritium disappeared in the conversion of the preparation into crystalline m-dinitrobenzene shows that it could not have been incorporated in the nitrobenzene, for even nitrobenzene-3-t would have lost much less.

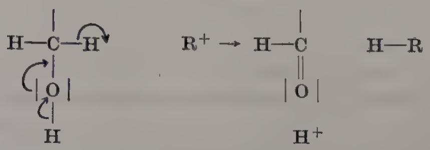
Table II and Table III show that the hydrogen attached to carbon atom 1 in the alcohol is the one that enters the aromatic nucleus, but the reaction velocity is dependent on the isotopic mass of the hydrogen. In the case of ethanol (Table II) the limits of error of $\beta_2 = k_T/k_H$ do not overlap for the two isomeric diazonium ions. From only these two results it is impossible to state that the two ratios are actually different, but if so, the difference would be of considerable interest for the discussion of the transition state of the reaction. In the case of methanol (Table III) the two values of o-nitrobenzenediazonium ion agree very well with one another and with that of the meta isomer.

The results parallel those of ALEXANDER and BURGE (2), who found that deuterium was introduced with a half to a sixth of the velocity of protium in the corresponding reduction with hypophosphorous acid. The effect should be less pronounced with deuterium than with tritium. KORNBLUM, COOPER, and TAYLOR (13) have shown that the latter reaction is probably a free radical chain reaction and proposed a mechanism, the hydrogen transference step of which is given as



$$\text{Aryl}\cdot + \left[\text{H—}\overset{\text{H}}{\underset{\text{O}}{\text{P}}}\text{—O}\right]^{-} \rightarrow \text{Aryl-H} + \left[\cdot\overset{\text{H}}{\underset{\text{O}}{\text{P}}}\text{—O}\right]^{-}$$

DEWAR (14) has proposed that the alcohol acts as a source of hydrogen anions in the reduction of diazonium salts. According to his views the compound Aryl-N=NH would be the first reduction product, which decomposes into Aryl-H and $N_2$. Whether this is so or the nitrogen is expelled at an early stage, leaving $\text{Aryl}^+$ or in some way Aryl· to accept the hydrogen, shall not be discussed here. It seems rather impossible that the ion $H^-$ should have any free existence in the acid medium, and it has been shown in this investigation that $H^+$ cannot be an intermediate.[1] In view of these facts and especially of the isotopic effect on the reaction rate the most natural assumption seems to be that the hydrogen is transferred in a single bimolecular process, the hydrogen never being free



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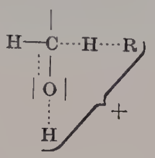
[1] These facts require the conversion of Aryl-N=NH into Aryl-H of Dewar to proceed via some intramolecular $\pi$-complex mechanism.

In this scheme $R^+$ symbolizes any of the two ions $ArylN_2^+$ or $Aryl^+$, and the alcohol acts in the sense of DEWAR (14). The reaction might be characterized as an $S_E2$ replacement on hydrogen. If the radical Aryl · is the acceptor, the hydrogen will be transferred together with only one electron.

In the transition state, which in the ionic case could be pictured



the hydrogen is bound to the carbon of the alcohol and to R. If the hydrogen is firmly bound in the transition state, the isotopic effect should be less pronounced than in a reaction with the atom essentially free in the transition state. A rate ratio of about 1: 7 for tritium and protium as in the present case can hardly be considered a strong effect even at 350° K (cf. 1 c).

In the mechanism pictured above, the lone electron pairs of the oxygen have been supposed to take part to a certain extent in the electronic mechanism. Their existence would be at least one of the reasons why hydrogen at carbon is so easily attacked when the carbon also carries the hydroxy group.

WESTHEIMER et al. (3, 15) have found that the rate-controlling step of the chromic acid oxidation of propanol-2 is an attack on the secondary hydrogen of the chromic acid ester of the alcohol. The picture resembles the present one. The question whether the alcohol even in this case reacts in the form of an (sulphuric acid) ester is open, but the concentration of ester cannot be very high in the medium in question.

## SUMMARY

Samples of methanol and ethanol carrying tracer amounts of tritium in different positions were prepared and used for the reduction of the diazonium salts corresponding to ortho- and meta-nitroaniline. It was shown that the hydrogens attached to carbon (atom 1) and oxygen, respectively, did not interchange appreciably under the conditions of the reactions, and that only the former replaced the diazonium group. From the specific activities of the alcohols and the deamination products the ratio of the reaction rates of tritium and protium could be calculated rather accurately. (The nitrobenzene was preferably converted into the easily purified meta-dinitrobenzene before the determination of tritium. According to previous investigations, nitrobenzene-3-t will lose exactly half of its tritium in this conversion.) Protium is found to react 7 times as fast as tritium. The results seem to be in agreement with the current conceptions of the reaction.

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REFERENCES. 1. **L. Melander,** Arkiv för Kemi *2* (1950) 211; a) p. 268, b) p. 258—260, c) section 7. — 2. **E. R. Alexander, R. E. Burge,** Jr., J. Am. Chem. Soc. *72* (1950) 3100. — 3. **F. H. Westheimer, N. Nicolaides,** J. Am. Chem. Soc. *71* (1949) 25. — 4. **J. W. Cornforth, G. Popják,** Nature *164* (1949) 1053. — 5. **K. E. Wilzbach, L. Kaplan,** J. Am. Chem. Soc. *72* (1950) 5795. — 6. **H. T. Clarke, E. R. Taylor** in **H. Gilman, A. H. Blatt,** Organic Syntheses, Coll.

Vol. I, 2nd ed., New York (1946), p. 415. — 7. **N. Kornblum,** Chapter 7 in **R. Adams** et al., Organic Reactions, Vol. II, New York (1944), p. 273. — 8. **L. Melander,** Acta Chem. Scand. *2* (1948) 440. — 9. **L. W. Covert, R. Connor, H. Adkins,** J. Am. Chem. Soc. *54* (1932) 1651. — 10. **F. G. Mann, B. C. Saunders,** Practical Organic Chemistry, London (1949), p. 39. — 11. **B. Eistert** in Neuere Methoden der Präparativen Organischen Chemie I, Berlin (1944), p. 396. — 12. **L. Orthner, L. Reichel,** Organisch-Chemisches Praktikum, 1. Aufl., Berlin (1929). — 13. **N. Kornblum, G. D. Cooper, J. E. Taylor,** J. Am. Chem. Soc. *72* (1950) 3013. — 14. **M. J. S. Dewar,** Research *3* (1950) 154. — 15. **F. Holloway, M. Cohen, F. H. Westheimer,** J. Am. Chem. Soc. *73* (1951) 65 and references.

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# Densities and boiling points of binary mixtures of acetone and some chlorinated ethanes

## By ARNE BRÄNDSTRÖM and LENNART SCHOTTE

With 4 figures in the text

In connection with other work we had reason to determine the densities and boiling point-composition curve for mixtures of acetone and some chlorinated ethanes. As these results are of sufficient accuracy to throw light on the behaviour of non ideal solutions in other connections, we shall give a short account of our measurements. These show a great deviation from the behaviour of an ideal solution for the boiling point-composition curve but only a slight deviation for the specific volumes.

*Specific volumes* ($v$) were determined for mixtures of acetone and 1.2-dichloroethane, 1.1.2-trichloroethane, and 1.1.1.2-tetrachloroethane respectively at a temperature of 25.1° by weighing to 0.1 mg a pycnometer containing about 10 ml of solution. The experimental values are given in Fig. 1 and a mathematical treatment (1) reduces these to the following functions:

For *acetone 1.2-dichloroethane*:

$$v = 10^{-2}(v_A \cdot c_A + v_E \cdot c_E) + 33 \cdot 10^{-8} \cdot c_A \cdot c_E$$

For *acetone 1.1.2-trichloroethane*:

$$v = 10^{-2}(v_A \cdot c_A + v_E \cdot c_E) - c_A \cdot c_E \cdot 10^{-7}\,[13.0 - 5.5 \cdot 10^{-2}(c_A - c_E)]$$

For *acetone 1.1.1.2-tetrachloroethane*:

$$v = 10^{-2}(v_A \cdot c_A + v_E \cdot c_E) - c_A \cdot c_E \cdot 10^{-7}\,[20.0 - 6.4 \cdot 10^{-2}(c_A - c_E)]$$

where $c_A$ and $c_E$ are the weight in grams of acetone and the chlorinated ethane respectively in 100 g of mixture ($c_A + c_E = 100$). $v_A$ and $v_E$ are the specific volumes of acetone and the chlorinated ethane respectively at the temperature mentioned.

The deviations, amounting to less than $2 \cdot 10^{-4}$ in the density, of the single values from these formulas can be found in Fig. 2, where the experimental values of the functions $\Delta v = 10^5\,[v - 10^{-2}(v_A \cdot c_A + v_E \cdot c_E)]$ are plotted against $c_A$. The curves represent the functions $\Delta v$. For the products used the following densities were found: acetone $d_4^{25.1} = 0.78412$, 1.2-dichloroethane $d_4^{25.1} = 1.24442$, 1.1.2-trichloroethane $d_4^{25.1} = 1.42705$, 1.1.1.2-tetrachloroethane $d_4^{25.1} = 1.54379$.

*The boiling point-composition curve* for the system acetone 1.2-dichloroethane was determined in an apparatus identical with that of SCATCHARD (2) with the exception of the thermal insulation. The temperature was measured with a

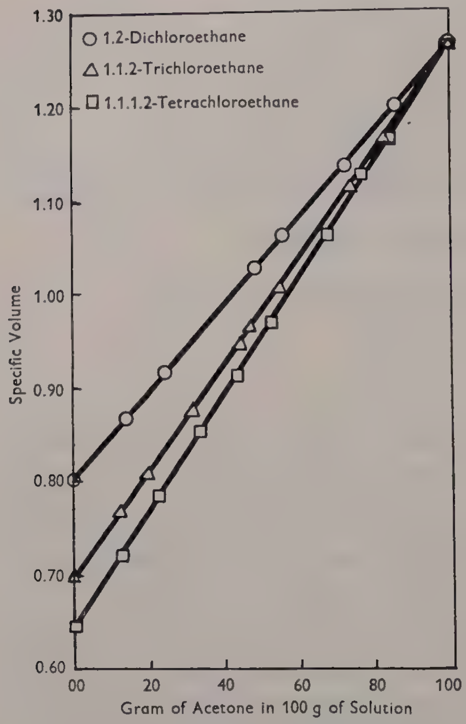

Fig. 1. Specific volumes of the systems: acetone-chlorinated ethanes.

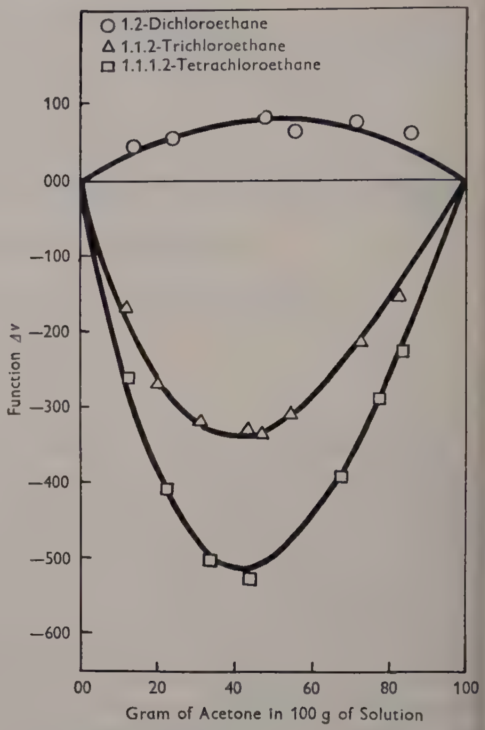

Fig. 2. Functions:
$\Delta v = 10^5 [v - 10^{-2} (v_A \cdot c_A + v_E \cdot c_E)]$

thermometer, graduated in tenths of a degree and corrected in the usual manner. The distillations were carried out at a pressure of 760.0 ± 3 mm and the boiling points were reduced to 760.0 mm. The pressure readings were made on a usual mercury barometer and corrected to standard conditions. The mole fractions were obtained from measurements of the densities.

In Fig. 3, the boiling points are plotted against the mole fractions of 1.2-dichloroethane. From the experimental values the following functions can be deduced:

For the liquid phase:

$$t_L = 56.13 + 27.43 \cdot x_1 - x_1 \cdot x_2 [6.0 - 2.2 (x_1 - x_2) - 2.6 (x_1 - x_2)^2]$$

For the vapor phase:

$$t_V = 56.13 + 27.43 \cdot x_1 + x_1 \cdot x_2 [13.2 - 7.8 (x_1 - x_2)]$$

where $t_L$ is the boiling point of a solution having the mole fractions $x_1$ and $x_2$ of 1.2-dichloroethane and acetone respectively. $t_V$ is the boiling point of a solution in equilibrium with a vapor having the mole fractions $x_1$ and $x_2$ of 1.2-dichloroethane and acetone respectively.

The deviations, amounting to less than 0.2°, of the single values, from these functions can be found in Fig. 4, where the experimental values of the function

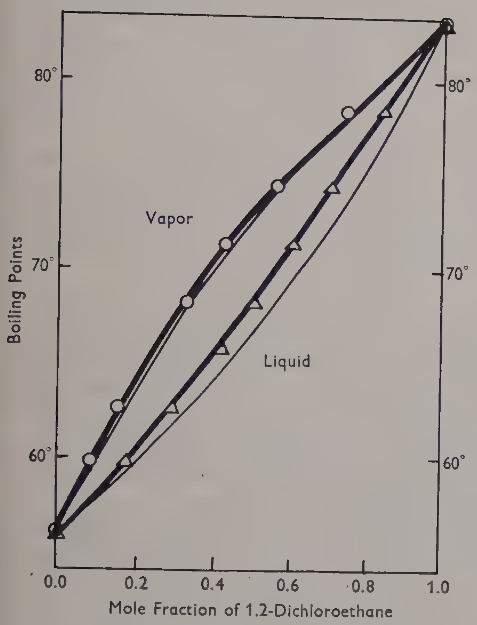

Fig. 3. Observed and calculated boiling point-composition curves for 1.2-dichloroethane-acetone mixtures.

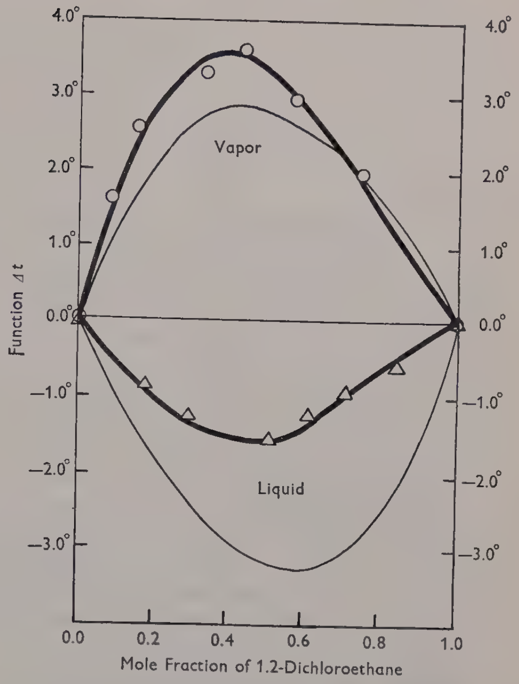

Fig. 4. Observed and calculated values of the functions:

$$\Delta t = t - 56.13 - 27.43 \cdot x_1$$

$\Delta t = t - 56.13 - 27.43\, x_1$, are plotted against $x_1$. The thick curves represent the functions $\Delta t$.

If the observed system is considered as being ideal, a theoretical boiling point-composition curve can be calculated from RAOULT's and DALTON's laws using the vapor pressures of both components. In this way the following equations were obtained:

For the liquid phase:

$$t_L = 56.13 + 27.43 \cdot x_1 - x_1 \cdot x_2 \left[12.7 + 3.8\,(x_1 - x_2) + 1.2\,(x_1 - x_2)^2\right]$$

For the vapor phase:

$$t_V = 56.13 + 27.43 \cdot x_1 + x_1 \cdot x_2 \left[11.0 - 3.8\,(x_1 - x_2) + 1.7\,(x_1 - x_2)^2\right]$$

The thin curves in Fig. 4 represent the functions $\Delta t$, calculated for an ideal solution.

The vapor pressures used for acetone were obtained from an excellent determination by ZMACZYNSKI (3), and we have collected his tabulated values in the following formula:

$${}^{10}\log p = 7.04771 - 1132.21\; 1/T - 79000\; 1/T^2$$

The agreement is very good (some hundredths of a per cent in $p$). However, the first one of his values is obviously in error (misprint?) and thus rejected. His boiling point for acetone, 56.131°, is in good agreement with ours. For 1.2-

dichloroethane the determinations of the vapor pressures are not so good. The values of PEARCE and PETER (4) seem to be the best ones and have been used for our calculation, but the boiling point for 1.2-dichloroethane, 83.94°, obtained from their formula given, is much too high in comparison with the accepted one, 83.5° — 83.6°, and the one we found, 83.56°. We assumed therefore that their thermometer was in error and all their boiling points were reduced accordingly.

A determination of the composition of the liquid and vapor phases of the system acetone-1.2-dichloroethane was carried out earlier by PAHLAVOUNI (5) but his equilibrium still was of a more primitive construction. His vapor phases were much richer in acetone than ours, probably due to some fractionating effects in his determinations.

**Reagents:** The *acetone* used was a pure technical product, dried over anhydrous potassium carbonate, distilled through a 80 cm Widmer column and a constant boiling middle fraction taken. The purity of the substance is obvious from the fact that the density of the condensed vapor and the liquid were the same within 0.00004.

The *1.2-dichloroethane* was a commercial product, distilled through a very efficient column and a fraction boiling within 0.02° taken. The authors are indebted to Dr. STINA STENHAGEN for this purification.

The *1.1.2-trichloroethane* was a commercial product, distilled through a 50 cm Widmer column and a middle fraction taken.

The *1.1.1.2-tetrachloroethane* was prepared according to PRINS (6) and distilled through a 30 cm Widmer column.

The authors are indebted to Professor STIG CLAESSON for valuable help and discussion.

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REFERENCES: 1. **Scatchard, G.** *Chem. Rev. 44,* 7 (1949). — 2. **Scatchard, G., Raymond, C. L.** and **Gilmann, H. H.** *J. Am. Chem. Soc. 60,* 1275 (1938). — 3. **Zmaczynski, A.** *J. Chim. Phys. 27,* 503 (1930). — 4. **Pearce, J. N.** and **Peter, P. E.** *J. Phys. Chem. 33,* 877 (1929). — 5. **Pahlavouni, E.** *Bull. Soc. Chim. Belg. 36,* 533 (1927). — 6. **Prins, H. J.** *Rec. Trav. Chim. 45,* 80 (1926).

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# Historical notes on the discovery of hafnium

By G. HEVESY

With 2 figures in the text

When I decided to choose the history of the discovery of hafnium as the subject of my contribution to the volume published in commemoration of Professor MANNE SIEGBAHN's 65th birthday, I did so in view of the fact that DIRK COSTER, one of his most eminent pupils, had such a great share in this discovery. He carried out his pioneer work at BOHR's institute shortly after terminating a year's stay at the Physics Laboratory in Lund.

Ever since the days of DALTON and BERZELIUS the atomic weight was by far the most outstanding concept of chemistry. The discovery of the atomic nucleus led to the introduction of another equally important concept, that of the atomic number[1], which was introduced by NIELS BOHR in 1913 (1). While the electronic constitution of the hydrogen atom, the helium and the lithium atom is described in this early paper, a comprehensive survey of the electronic constitution of all members of the periodic system was given by him in a paper, entitled "Der Bau der Atome und die physikalischen und chemischen Eigenschaften der Elemente" (2), published 9 years later. The leading idea of this work was to follow the building-up of the electron groups in the atom by successive addition of electrons, one by one, using the information regarding the binding process of each electron provided by the structure of the accompanying spectrum. In this paper BOHR has shown among others that during the building-up of the group structures it happens sometimes that electrons in a subgroup with given quantum numbers are more firmly bound than in subgroups of lower principal number, but higher subordinate number. The former subgroups therefore appear in the atom before the latter, but with increasing atomic number the normal relationship between the strength of the various types of electron binding is restored and the groups of lower principal quantum number are filled up, while the development of higher principal groups is brought to a temporary standstill. This accounts for the anomalous positions within the periodic table of such families of elements as the rare earths, which are due to a temporary delay in regular development of outer electron groups with increasing atomic number, caused by some transitory stage in the development of inner groups. According to BOHR's theory, all such transitory stages in the regular development of the atomic group structures were now simply accounted for by addition of new subgroups to principal electron groups that are only partially completed, and the apparent irregularities in the periodic table appear as a direct consequence of quite elementary features of the quantum theory. As, according to BOHR's theory, the subgroup of electrons which characterizes the rare earth elements contains 14 electrons only, the number of rare earth elements is also 14 and thus includes the elements starting with the element 58 (cerium) and ending with the element 71 (lutetium). Correspondingly, the element 72 must be a number of the next vertical group of the periodic table, that of titanium. BOHR's paper contains an abundance of results of outstanding interest both for the physicist and the chemist so that such an important statement as the one that the missing element 72 must be a member of the titanium group found place in a foot-note only. Though so unconspicuously located, the remark did not evade careful scrutiny characterizing all of BOHR's statements. He became cognizant of the fact that URBAIN, when crystallizing a solution containing the element 71, found 23 not earlier observed spectral lines, and he ascribed the appearance of these lines to the presence of a new element, that of the atomic number 72, which thus should be a member of the rare earth group. Furthermore, DAUVILLIER, when investigating the x-ray spectrum of URBAIN's sample, found two weak lines which he ascribed to the presence of the element 72.

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[1] As early as in 1897, RYDBERG pointed out that in theoretical consideration it is not the atomic weight, but the ordinal number of the element which is to be considered.

In the last week of May 1923, NIELS BOHR consulted COSTER, who came from Lund for a short visit, and myself who was attached to his institution, whether in view of URBAIN's and DAUVILLIER's findings he rather should change or possibly omit his remark on the nature of the element 72. While COSTER, through his Lund studies, had become an expert in the field of x-ray spectroscopy, I was far from being an expert in the field of the chemistry of rare earth elements. However, in view of the fact that I had been invited by MOSELEY to assist him in the study of the x-ray spectrum of the last members of the rare earth group and only by the outbreak of the first world war was prevented from joining him, I had studied the literature dealing with the chemistry of the rare earth elements. I agreed with COSTER in that the findings of URBAIN and of DAUVILLIER in no way disprove the correctness of BOHR's prediction as to the nature of the element 72. In view of the great complexity of the optical spectrum of the rare earth elements, the appearance of new spectral lines during a concentration process cannot be considered a proof of the presence of a yet unknown element in the sample investigated. In fact, after the discovery of the element 72, HANSEN and WERNER succeeded in showing that URBAIN's 23 new spectral lines were due to the presence of lutetium in his sample (3). Nor can the two weak x-ray lines observed be considered to prove the identity of URBAIN's "celtium" lines with the missing element 72. How unjustified the claim of the discovery of a new element based on the appearance of two weak x-ray lines can be was shown later, when the element 43 (masurium) was claimed to be discovered on such a basis: as we now know, masurium is not found in nature and can be artificially produced only.

After our talk with NIELS BOHR we did not pursue the subject and none of us thought at that date to initiate a search for the element 72. It was a coincidence of fortunate events which led to the discovery of the element, the character of which was predicted by BOHR, in his institute. Among these events COSTER's stay in Copenhagen after the completion of his studies with MANNE SIEGBAHN at Lund was the most important one. In collaboration with COSTER we intended to use radiolead as anode of an x-ray tube and to investigate the possible effect of intense and prolonged irradiation on the decay rate of radium D, which should reflect itself in a corresponding change in the rate of decay of radium E. While, in search of a suitable high-voltage aggregate, being much interested in the application of x-ray spectroscopy to mineral chemistry, I suggested that COSTER should look for the element 72. In the beginning he was very reluctant to embark on this investigation, maintaining the view that, if element 72 was present in a mineral, its concentration could be expected to be only very low and, since the sensitivity of x-ray spectroscopy in those days was rather weak, it seemed almost hopeless to discover the missing element by means of this method. Finally, however, COSTER yielded to my argument that the aim of our work should be to enable me to learn the technique of x-ray spectroscopy and we could just as well at the same time try to find BOHR's element 72.

As in the periodic table the element 72 has an intermediary position between zirconium and thorium, it could be present both in zirconium and in thorium minerals. Most fortunately we decided to seek the missing element in zirconium minerals. As we found later, most thorium minerals do not contain detectable amounts of the element 72 and our search in thorium minerals would have been abortive.

The first x-ray spectrum taken by COSTER was that of a concentrate of a Norwegian zircon. It showed both the $L\alpha_1$ and $L\alpha_2$ lines of the element 72. We then focussed our interest on the x-ray spectrum of commercial zirconium oxide samples of different origin. All these samples exhibited the presence of the $L\alpha$ doublet of the element 72, which was estimated to be present in a concentration of about 1 per cent. This omnipresence of the $L\alpha$ doublet was rather embarrassing in view of the fact that the zirconium $K\alpha$ doublet in second order happens to coincide with the $L\alpha$ doublet of hafnium. It is quite easy to distinguish between the K lines of zirconium and the much softer L lines of hafnium, for example, by keeping the tube voltage below the excitation voltage, but with the aggregate at our disposal it was difficult to ensure that we actually remained below that level. The final proof of the discovery of hafnium was brought when COSTER succeeded in locating the $\beta_1$ and $\beta_2$ lines and soon was able to determine the whole x-ray spectrum of that element. On the 11th of December 1922 a few minutes only before BOHR started his Nobel lecture in Stockholm which I had the privilege to attend, COSTER announced by telephone the presence of the $Hf\beta_1$ and $Hf\beta_2$ lines on his photographic plate. BOHR mentioned the discovery of the element 72 in his lecture.

In the years to come, we determined the hafnium content of hundreds of zirconium containing minerals. They all contained hafnium, the zirconium/hafnium ratio varying between 1 000 and 10 and, in a sample of the rare scandium mineral thortveitit, the hafnium oxide content amounted to almost that of the zirconium oxide content. The terrestric abundance of hafnium was found to be $5 \times 10^{-6}$ g per g igneous rock, 1/200 000 of the earth crust being thus composed of hafnium, while $1 \times 10^{-6}$ g was found in 1 g of stony meteorites.

Shortly after the discovery of hafnium I studied MARIGNAC's papers on the preparation and properties of the double fluorides of zirconium. The reading of this fine work of MARIGNAC induced me to attempt to separate hafnium from zirconium by fractional crystallization of ammonium, zirconium fluoride resp.

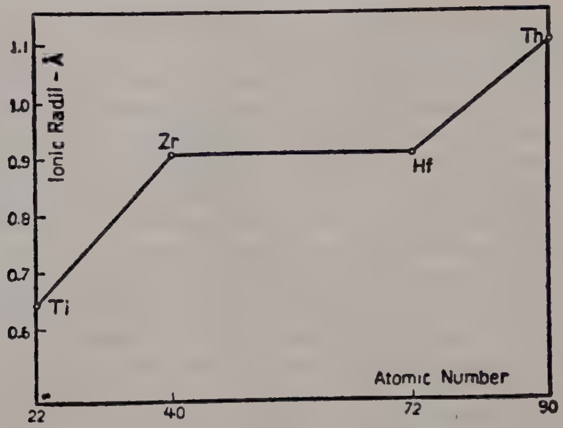

Fig. 1. Ionic sizes of the oxides.

potassium zirconium fluoride. This work involved the use of several large platinum dishes, as great amounts of the zirconium salt were to be crystallized. JULIUS THOMSEN, presumably through his affiliation to the Greenland cryolite mines, became interested in the chemistry of fluorides and purchased several large platinum dishes and beakers at the remarkable price of 60 öre per gram. These platinum dishes were very kindly put at our disposal by JULIUS THOMSEN'S successor, Professor BILMANN. The fractional crystallization of hafnium containing ammonium zirconium fluoride proved to be very successful due to the relatively large difference in the solubilities of the hafnium and zirconium ammonium hexafluorides. All commercially available hafnium compounds sold till the outbreak of the war were prepared by the Deutsche Gasglühlicht Auer Gesellschaft making use of this method.

When, a few years later, I met the great rare earth chemist AUER VON WELSBACH who took great interest in our work, he expressed his astonishment that we prepared hafnium by crystallizing such unpleasant compounds as the fluorides instead of working up the sulphates which he applied with so much success. The sulphates of hafnium and those of zirconium were later found by us to have almost identical solubilities. Thus, crystallization of these compounds would not have led to the separation of hafnium from zirconium. Luck favoured us, when we concentrated on the fractional crystallization of fluorides, as in many other phases of our work on the element 72.

As all zirconium contains appreciable amounts of hafnium, one would expect that all values obtained for the atomic weight of zirconium before the discovery of hafnium were too high. Before the introduction of RICHARD'S methods which are based on the distillation of halogenates, the atomic weight of zirconium was determined by the sulphate method. After weighing the sulphate the sample was ignited and the weight of the oxide obtained determined. As some sulphate is retained by the oxide, too low values are obtained for the atomic weight of zirconium when the atomic weight is calculated from the weight ratio of the sulphate and the oxide. This error compensated, mostly even over-compensated, the error in the atomic weight determination of zirconium due to the presence of hafnium. A correct value for the latter was obtained by HÖNIGSCHMID who

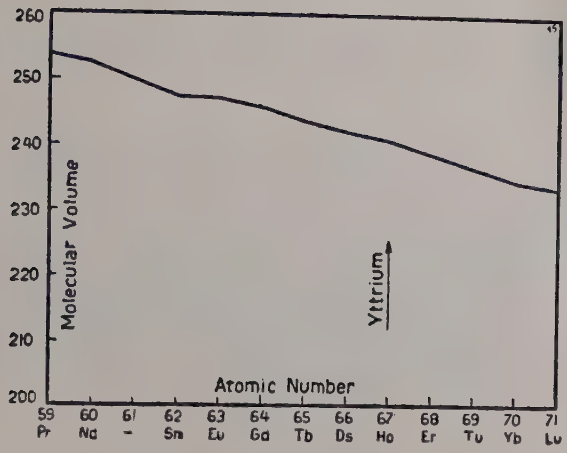

Fig. 2. Lanthanide contraction.

made use of our zirconium samples, purified from hafnium, which he analysed as volatile chlorides. Venable, on the other hand, who several years before the discovery of hafnium analysed the volatile bromides, applying thus a correct method, obtained a much too large figure for the atomic weight of zirconium. For several years he was resultlessly engaged in the elucidation of this discrepancy. After the discovery of hafnium he asked us to determine his zirconium samples for their hafnium content. By making use of the figures obtained by us he could then correct his atomic weight values for the presence of hafnium. The value now obtained for the atomic weight of zirconium was the same as that found by Hönigschmid.

Pure hafnium compounds were used for the determination of the density of hafnium oxide which then was compared with that of zirconium oxide. The molecular volumes of zirconium oxide and hafnium oxide, and thus the size of the zirconium and hafnium ion, were found to be almost identical (Fig. 1). They differ, as found later by Böhm (4), with 0.5 per cent only. In contrast the molecular volume of lanthanum oxide (57) is appreciably larger than that of yttrium oxide (45), from this fact I concluded that the appearance of the rare earth group has to lead to a contraction of the molecular volume; this conclusion induced me to determine the change of the molecular volume when passing from one rare earth element to another. This was done by measuring the density of the isomorphous octohydrosulphates of the various rare earth samples kindly presented me by Auer von Welsbach. The result of these measurements, shown in Fig. 2, clearly demonstrates the contraction of the molecular volume and correspondingly that of the ionic radius when passing from one element of the rare earth group to the next. This "rare earth contraction" was simultaneously observed by Goldschmidt (6) who, in his classical investigations on the rules of geochemical distribution, determined the ionic radii of the rare earth elements and denoted the above "rare earth contraction" as "lanthanide contraction".

It follows from Bohr's theory that the above mentioned contraction is to be expected in all those parts of the periodic system in which the groups of lower principal quantum number are filled up, in the iron group, the platinum group, the rare earth group, and the transuranium group. The existence of the last one being predicted by BOHR and discovered in recent years in a most spectacular way at Berkeley.

When expressing my most hearty wishes to Professor MANNE SIEGBAHN for the years to come I want simultaneously to be permitted to lower my flag before the memory of his eminent pupil, DIRK COSTER, the distinguished physicist and chevalier sans peur et sans reproche.

REFERENCES. (1) **N. Bohr,** *Phil. Mag., 26,* 857 (1913). — (2) **N. Bohr,** *Z. f. Phys., 9,* 1 (1922). — (3) **H. M. Hansen** and **S. Werner,** *Kgl. Danske Vid. Selskab. Math. Fys. Medd., V,* 8 (1923). — (4) cf. **G. Hevesy,** Chemical Analysis by X-Rays and Its Applications, *Mc Graw-Hill Book Comp.* (1932). — (5) **G. Hevesy,** *Z. anorg. Chem., 147,* 217 and *150,* 68 (1925). — (6) **V. M. Goldschmidt, B. Ulrich** and **Th. Barth,** *Skrifter Norske Videnskaps Akad., Oslo, Matemat. Naturw. Kl. No. 5* (1925).

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# On the growth substance activity of enantiomorphic α-(naphthoxy)-propionic acids

By BÖRJE ÅBERG

With 2 figures in the text

Already in 1935 several synthetic substances exerting many of the physiological properties of the native auxins were described, and since then their number has been steadily increasing. Apparently there must be some common structural properties which enable these substances to act as growth regulators, and much work has been done to find out the minimum structural requirements for activity. An excellent review of this work has recently been given by THIMANN (6). Here we shall only consider the question of differential activity of optical isomerides which was first raised by KÖGL in 1937 (4). He found (+)-α-(3-indolyl)-propionic acid to be 30 times more active in the standard Avena curvature test than the antipode. Later he reports (5) that this difference is absent in the Avena cylinder test (straight growth), and concludes that it is not related to the primary activity but rather to the auxin transporting mechanisms. Recently THIMANN (6) reported (+)-α-(2,4-dichlorophenoxy)-propionic acid to be two times as active in the pea test as the racemic mixture, and referred to experiments by VELDSTRA showing much higher activity for (—)-1,2,3,4-tetrahydronaphthoic acid in the pea test than for the antipode. He concluded that there must also be differences in the primary activity of optical isomerides.

On the suggestion of Professor ARNE FREDGA, Uppsala, I started some experiments with the enantiomorphic forms of α-(1-naphthoxy)-propionic acid in June 1950 which were later extended also to the α-(2-naphthoxy)-propionic acids. (In the following the dextro-rotatory forms are denoted (+)-1-NOP and (+)-2-NOP, the laevo-rotatory forms (—)-1-NOP and (—)-2-NOP resp.) The root growth test already used for the study of auxin antagonists and synergists (1) was utilized. In this test the contact of the reacting cells with the applied substances is even more intimate than in the pea test or the Avena cylinder test, and the differences observed thus probably refer to their primary action.

After the conclusion of the experiments the recent paper of WAIN (8), reporting much higher activity for (+)-2-NOP than for the (—)-form, came to my attention. As different test methods were used, the data here presented for 2-NOP not only corroborate WAIN's results but also permit some further conclusions as to the different activity of the enantiomorphic forms.

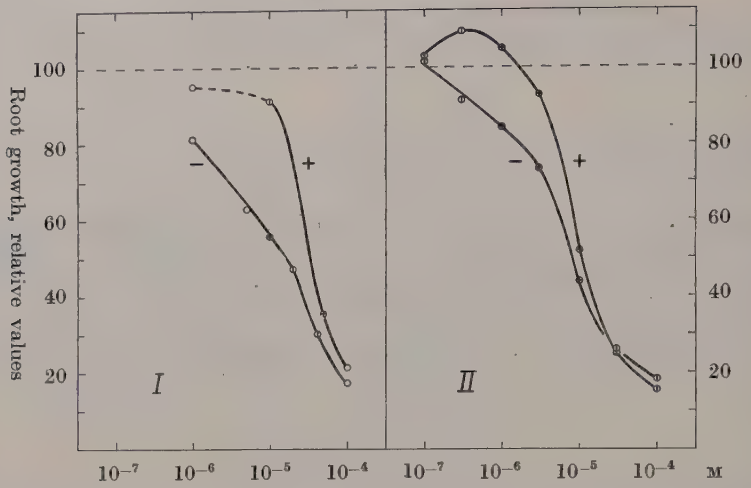

Fig. 1. The effects of (+)- and (—)-1-NOP on root growth. I: June — Aug. 1950, II: March 1951. Root growth in per cent of control plotted against a logarithmic concentration scale (M = mole per litre). Number of tubes indicated as in fig. 2.

## Methods

Flax seedlings, grown for 28 hours on moist filter paper, were transferred to cork holders, floating on the test solutions, where they remained for 17 hours at 25° C. The new growth was then measured. The solutions contained a phosphate buffer (6.7 mM), 5 mM Ca-nitrate and the substance to be tested; the pH was maintained as close to 5.9 as possible. The test is identical with the *S*-test described in an earlier communication (1), where further details can be found.

## Experimental results

The activity of the (+) and (—)-forms of 1-NOP has been tested on two occasions (fig. 1). The results are of quite the same type, the activity in repressing root growth being somewhat higher for both forms during the second period, however. This phenomenon is probably due to a slight variation in the sensitivity of the roots, which, though usually of inconsiderable magnitude, cannot be wholly avoided. This variation is naturally at a minimum, when the different substances are compared in the same experimental series. Results of such experiments are presented in table 1, and they compare favourably to the curves of fig. 1:II which represent also some other series.

Thus, it seems certain that (—)-1-NOP at medium concentrations possesses higher activity in depressing root growth than the (+)-form, and that this difference becomes gradually smaller at higher or lower concentrations due to the different form of the activity curves. The stimulative effect of low concentrations of (+)-1-NOP is consistent for the experiments of group II: $10^{-6}$ M gives a relative growth rate of 101—110 % (mean for 13 tubes, each with 14 seedlings, 104.9), and $3 \times 10^{-7}$ M 107—112 % (mean for 9 tubes 109.3). The

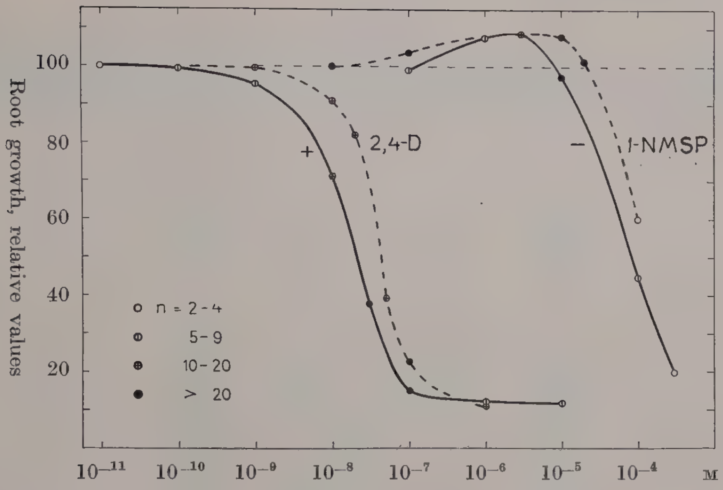

Fig. 2. The effects of (+)- and (−)-2-NOP on root growth. The activity curves of 2,4-D and 1-NMSP are also shown for the sake of comparison. 1-NMSP $10^{-6}$ M: 108.2 % ($n = 30$), $3 \times 10^{-6}$ M: 108.3 % ($n = 10$). $n$ is the number of tubes each with 14 seedlings, represented by the points.

*Table 1*

*S*-tests with (+)-1-NOP and (—)-1-NOP. Each of the absolute values represents the average growth of 14 roots. The exponential numbers denote the molar concentrations.

| | Exp. | Control | | 1-NOP | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | $10^{-5}$ | $3 \times 10^{-6}$ | $10^{-6}$ | $3 \times 10^{-7}$ | $10^{-7}$ |
| Growth, mm/17 h | *1* | 21.8<br>22.5<br>23.8 | (+) | | 21.5<br>21.9<br>23.1 | 24.3<br>25.2<br>25.3 | 24.2<br>25.9<br>26.2 | 21.6<br>23.9<br>25.2 |
| | | | (−) | | 15.0<br>15.8<br>16.5 | 16.6<br>18.6<br>18.8 | 19.4<br>19.7<br>21.7 | 21.8<br>22.4<br>24.6 |
| | *2* | 22.8<br>25.2<br>25.3 | (+) | 12.9<br>13.2<br>13.3 | 22.7<br>23.4<br>23.7 | 25.8<br>25.9<br>26.6 | 25.6<br>26.0<br>27.1 | |
| | | | (−) | 9.6<br>10.2<br>10.4 | 17.6<br>17.7<br>18.6 | 19.4<br>20.2<br>21.2 | 21.6<br>21.8<br>24.7 | |
| Growth, relative values | *1* | 100 | (+) | — | 98 | 110 | 112 | 104 |
| | | | (−) | — | 69 | 79 | 89 | 101 |
| | *2* | 100 | (+) | 54 | 95 | 107 | 107 | — |
| | | | (−) | 41 | 74 | 83 | 93 | — |

*Table 2*

*S*-tests with (+)-2-NOP and (—)-2-NOP. Values presented as in table 1.

| | Exp. | Control | $10^{-5}$ (—) | $3\times10^{-8}$ (+) | $3\times10^{-8}$ (+) $+10^{-5}$ (—) | $10^{-7}$ (+) | $10^{-7}$ (+) $+10^{-5}$ (—) |
|---|---|---|---|---|---|---|---|
| Growth, mm/17 h | *1* | 23.4 | 21.4 | 7.6 | 16.5 | 3.9 | 9.6 |
| | | 24.8 | 21.8 | 7.6 | 17.3 | 3.9 | 10.6 |
| | | 24.9 | 22.3 | 7.7 | 17.8 | 4.4 | 11.2 |
| | | 25.2 | 22.9 | 8.2 | 18.1 | 5.0 | 11.5 |
| | *2* | 24.9 | 23.4 | 8.1 | 16.9 | 4.0 | 11.4 |
| | | 25.4 | 23.4 | 8.9 | 17.7 | 4.1 | 12.4 |
| | | 25.6 | 23.8 | 8.9 | 18.7 | 4.8 | 12.7 |
| | | 26.1 | 24.4 | 9.1 | 18.9 | 4.8 | 13.1 |
| Growth, relative values | *1* | 100 | 90 | 32 (100) | 71 (224) | 18 /100/ | 44 /250/ |
| | *2* | 100 | 93 | 34 (100) | 71 (206) | 17 /100/ | 49 /280/ |

value for $10^{-6}$ M in group I represents only 2 tubes, and cannot be taken as evidence against such a stimulative action.

The activity curves of the 2-naphthoxypropionic acids are shown in fig. 2. It is apparent at the first sight that the difference between the enantiomorphic forms is here much greater and, further, that the signs are reversed, the (+)-form being about 3,000 times more active in repressing root growth than the (—)-form. The effect of (+)-2-NOP is in fact quite comparable to that of 2,4-dichlorophenoxyacetic acid (2,4-D) or indole-3-acetic acid (IAA), the concentrations needed for 50 % inhibition in the *S*-test being as follows: IAA $1.1\times10^{-8}$, (+)-2-NOP $2.1\times10^{-8}$ and 2,4-D $3.8\times10^{-8}$ M. The (—)-form, on the other hand, compares well to an auxin antagonist like α-(1-naphthylmethylsulfide)-propionic acid (1-NMSP, see 1, 2).

Under the circumstances it was pertinent to test the (—)-form as an antagonist to the growth-depressing activity of (+)-2-NOP. As is apparent from table 2 $10^{-5}$ M (—)-2-NOP has in fact a conspicuous growth-restoring effect upon roots growing in $3\times10^{-8}$ or $10^{-7}$ M (+)-2-NOP, the growth rate being more than doubled by such an addition. This interrelation is not restricted to the antipodes, however, as the (—)-form antagonizes the effects of 2,4-D and the effects of (+)-2-NOP are diminished by 1-NMSP. In the first case the effects of two different concentrations of (—)-2-NOP upon roots growing in $10^{-7}$ M 2,4-D were tested (table 3), and in the second case $2\times10^{-5}$ 1-NMSP was added to $3\times10^{-8}$ or $10^{-7}$ M (+)-2-NOP (table 4).

As earlier shown (1, p. 458) the "activity" of a growth substance in the presence of an antagonist may be roughly estimated from the standard activity curve of this substance, and its "activity coefficient" (*A*) may be calculated. The coefficients calculated from tables 2–4 have been compiled in table 5 together with some data from (1) and from unpublished experiments. As may be expected the *A*-values for a certain concentration of a growth substance diminish with increasing concentrations of the antagonist, and, with due respect to this circumstance, the effect of (—)-2-NOP seems to be of the same mag-

*Table 3*

*S*-tests with 2,4-D and (—)-2-NOP. Values presented as in table 1.

|  | Exp. | Control | (—)-2-NOP |  | $10^{-7}$ 2,4-D + (—)-2-NOP: |  |  |
|---|---|---|---|---|---|---|---|
|  |  |  | $3\times10^{-6}$ | $10^{-5}$ | 0 | $3\times10^{-6}$ | $10^{-5}$ |
| Growth, mm/17 h | *1* | 23.1<br>23.5<br>24.1<br>24.1<br>25.2 | 24.6<br>26.0<br>26.1<br>26.6<br>27.3 | 24.0<br>24.6<br>25.1<br>26.2<br>26.3 | 5.1<br>5.4<br>5.6<br>5.8<br>6.4 | 9.4<br>10.0<br>10.6<br>10.7<br>11.1 | 12.8<br>12.8<br>13.4<br>13.7<br>14.2 |
|  | *2* | 23.1<br>24.3<br>25.0<br>25.6<br>25.7 | 25.8<br>26.1<br>27.2<br>27.3<br>28.1 | 23.7<br>24.5<br>26.2<br>26.3<br>27.0 | 4.9<br>5.1<br>5.2<br>5.6<br>5.9 | 9.8<br>10.8<br>10.8<br>10.9<br>12.4 | 13.3<br>13.5<br>14.0<br>14.1<br>14.3 |
| Growth, relative values | *1* | 100 | 109 | 105 | 24 (100) | 43 (183) | 56 (236) |
|  | *2* | 100 | 109 | 103 | 22 (100) | 44 (205) | 56 (259) |

*Table 4*

*S*-tests with (+)-2-NOP and 1-NMSP. Values presented as in table 1.

|  | Exp. | Control | 1-NMSP $2\times10^{-5}$ | $3\times10^{-8}$ (+) + 1-NMSP: |  | $10^{-7}$ (+) + 1-NMSP: |  |
|---|---|---|---|---|---|---|---|
|  |  |  |  | 0 | $2\times10^{-5}$ | 0 | $2\times10^{-5}$ |
| Growth, mm/17 h | *1* | 25.4<br>25.7<br>26.5<br>26.8<br>26.8 | 24.4<br>24.9<br>25.3<br>26.0<br>27.1 | 10.1<br>10.5<br>10.7<br>11.1<br>11.4 | 20.8<br>21.0<br>21.2<br>22.3<br>22.6 | 4.4<br>4.6<br>4.8<br>4.9<br>5.0 | 15.2<br>15.2<br>15.3<br>15.4<br>15.9 |
|  | *2* | 25.8<br>26.3<br>26.3<br>26.6<br>26.8 | 24.8<br>25.7<br>26.2<br>26.2<br>26.9 | 8.8<br>9.4<br>10.7<br>11.0<br>11.5 | 21.6<br>21.7<br>22.5<br>22.9<br>22.9 | 3.8<br>4.2<br>4.6<br>4.8<br>5.0 | 15.6<br>15.9<br>16.4<br>16.5<br>17.2 |
|  | *3* | 25.4<br>25.7<br>26.1<br>26.1<br>28.1 | 23.7<br>24.3<br>24.8<br>25.3<br>25.8 |  |  | 3.9<br>4.2<br>4.4<br>4.7<br>4.9 | 14.7<br>15.3<br>15.7<br>16.1<br>17.1 |
| Growth, relative values | *1* | 100 | 97 | 41 (100) | 82 (200) | 19/100/ | 59/325/ |
|  | *2* | 100 | 99 | 39 (100) | 85 (217) | 17/100/ | 62/364/ |
|  | *3* | 100 | 94 | — | — | 17/100/ | 60/357/ |

*Table 5*

Activity coefficients ($A$) of some growth substances in the presence of (—)-2-NOP or 1-NMSP. The exponential numbers denote the molar concentrations.

| Antagonist | Growth substance | | | | | |
|---|---|---|---|---|---|---|
| | (+)-2-NOP | | 2,4-D | | NAA | IAA |
| | $3 \times 10^{-8}$ | $10^{-7}$ | $5 \times 10^{-8}$ | $10^{-7}$ | $10^{-7}$ | $10^{-7}$ |
| (—)-2-NOP, $3 \times 10^{-6}$ | — | — | — | 0.45 | — | — |
| ,, $10^{-5}$ | 0.33 | 0.23 | — | 0.35 | — | — |
| 1-NMSP, $3 \times 10^{-6}$ | — | — | — | 0.45 | — | — |
| ,, $10^{-5}$ | — | — | — | 0.28 | 0.28 | — |
| ,, $2 \times 10^{-5}$ | 0.18 | 0.15 | 0.26 | 0.18 | 0.20 | 0.22 |

nitude as that of 1-NMSP. A more curious phenomenon which has been found also in more extensive series with 1-NMSP and 2,4-D, is, that for a given concentration of the antagonist the $A$-value diminishes with rising growth substance concentration, i.e. with decreasing quotient antagonist/growth substance. The explanation of this fact is not quite clear, but it is possible that a slight toxic effect of the antagonist may interfere more strongly with the results at the lower auxin concentrations, thus causing the discussed trend of the $A$-values.

## Discussion

From figs. 1 and 2 it is apparent that in respect to the growth effects (+)-1-NOP is more nearly related to (—)-2-NOP than to (+)-2-NOP. There are chemical grounds also for believing that the absolute configuration of (+)-1-NOP is related to (—)2-NOP (3), and it is natural that the physiological activity should follow this configuration rather than the sign of the specific rotation. For (—)-2-NOP it has been shown that it acts as an auxin antagonist in root growth (tables 2–5) and that in this respect it is quite comparable to the earlier studied 1-NMSP (1). In different tests involving shoot parts it has been found to posses negligible auxin activity (8). The activity curve of (+)-1-NOP, with conspicuous stimulative effects at low concentrations, is of the type found for (—)-2-NOP, 1-NMSP and several related auxin antagonists, and it seems highly probable that it should exert an antagonistic action to externally applied auxins, though direct evidence and quantitative data are not yet available.

Judged from the effects upon root growth (+)-2-NOP is an auxin with an activity intermediate to that of IAA and 2,4-D. It has also been shown to be highly effective in the Avena curvature test, the Avena cylinder test and the pea test, as well as in inducing parthenocarpy and rooting responses (8), so there can be no doubt as to its auxin activity. (—)-1-NOP, on the other hand, has rather weak effects upon root growth with an activity curve which comes close to that of a hemi-auxin like cyclohexaneacetic acid. It should be premature, however, to classify it among the hemi-auxins before further details about its physiological effects are known.

That the auxin activity of naphthoxyalkylcarboxylic acids is enhanced by placing the side chain in 2-position rather than in 1-position has been earlier shown by VELDSTRA (7, p. 115) for the naphthoxyacetic acids: the 2-derivative shows an activity of 60 % in the pea test, while that of the 1-derivative is only 2 % of that of IAA. It is worthy of notice that the reverse is true for the naphthaleneacetic acids.

The most important fact revealed by the present investigation is that a typical auxin can be converted into an auxin antagonist by a change in the configuration of an asymmetric carbon atom in the side chain. Such highly specific conditions are not included in the structural requirements for auxin activity as hitherto formulated, and are certainly apt to shed further light over this question as well as over the nearly related problem of the requirements for anti-auxin activity.

## SUMMARY

The enantimomorphic forms of α-(1-naphthoxy)-propionic acid [(+)-1-NOP, (—)-1-NOP] and of α-(2-naphthoxy)-propionic acid [(+)-2-NOP, (—)-2-NOP] have been tested in a root growth test, using flax seedlings growing in buffered test solutions. (+)-2-NOP acts like an effective auxin in depressing root growth, while (—)-2-NOP behaves as an auxin antagonist. The difference between (+)-1-NOP and (—)-1-NOP is conspicuous though much less pronounced and of different sign, (+)-1-NOP being physiologically related to (—)-2-NOP.

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**REFERENCES.** 1. **B. Åberg,** Physiologia Plantarum *3,* 447 (1950). — 2. **A. Fredga,** Ark. f. Kemi *3* (16), 123 (1951). — 3. **A. Fredga & M. Matell,** Ark. f. Kemi *3* (47) 429 (1951). — 4. **F. Kögl,** Naturwiss. *25,* 465 (1937). — 5. **F. Kögl & B. Verkaaik.** Z. physiol. Chem. *280,* 167 (1944). — 6. **K. V. Thimann,** in **F. Skoog,** Plant growth substances, Wisc. 1951, p. 21. — 7. **H. Veldstra,** Enzymologia *11,* 97 (1944). — 8. **R. L. Wain,** Jour. Sci. Food & Agric. *2* 101 (1951).

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# Bildung und Spaltung von Nitro-Verbindungen. I

Von HANS VON EULER, HANS HASSELQUIST und INGRID LOEPER

Mit 3 Figuren im Text

## A. Bemerkungen über die Ionen Nitronium und Phosphonium

Die Problemstellungen, die den hier mitzuteilenden Versuchen zugrundeliegen, gehen aus von unseren Erfahrungen über die Bildung und Spaltung von aromatischen und aliphatischen Nitro-Verbindungen. Nachdem die Hypothese des einen von uns (H. v. E.[1]), dass die Nitrierung von Benzol in schwefelsaurer Lösung unter Vermittlung des Nitronium-Ions, $NO_2^+$, erfolgt, durch die grundlegenden reaktionskinetischen Arbeiten von C. K. INGOLD[2]) und seiner Schule bewiesen war, hat sich gezeigt (EULER u. HASSELQUIST C. r. 233, 800; 1951), dass auch die Nitrierung anderer Molekülarten an Bedingungen gebunden ist, unter welchen das Nitronium-Ion zur Wirkung kommt, also in Gemischen von starken Säuren mit Salpetersäure, oder bei Anwesenheit von Verbindungen, welche, wie Nitrosylchlorid oder $N_2O_5$ oder daraus dargestellte Salze (Soc. 1950, 2559) oder Dissociation das Nitronium-Ion liefern (INGOLD[3]); KHARASCH u. MITARB.[4]). Man kann die erwähnte Nitrierungsreaktion so beschreiben, dass durch die Gegenwart von starken Säuren (Perchlorsäure; Dischwefelsäure, GILLESPIE) oder von Eisessig die elektrolytische Dissociation der Salpetersäure in $H^+$ und $NO_3^-$ zurückgedrängt, und dementsprechend die Dissociation in $HO^-$ und $NO_2^+$ erhöht wird. Im System Salpetersäure-Schwefelsäure erfährt der Benzolkern eine Anregung. — Addition an unges. Kohlenwasserstoffe, Rec. 70, 689.

Auch in der heterocyklischen Serie wird die Nitrierung offenbar durch das Nitronium-Ion vermittelt (rauchende Salpetersäure bezw. konz. Salpetersäure mit Schwefelsäure oder Eisessig); sie verläuft aber keineswegs immer so glatt wie in der arom. Reihe; Pyridin, Thiophen u. a. fordern hohe Nitrierungstemperaturen.

*Furan* wurde von MARQUIS[5]) mit rauchender Salpetersäure- Essigsäureanhydrid bei 5° nitriert. Er erhielt aus Petroleumäther hellgelbe Krystalle vom Schmpt. 28°, leicht löslich in organischen Lösungsmitteln, wenig löslich in Wasser und mit gelbbrauner Farbe in Alkali.

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[1] EULER, Lieb. Ann. 330, 280 u. zw. 286; (1903). — Diese Auffassung wurde später auch von W. OSTWALD vertreten. Grundriss d. allgem. Chemie, 4. Aufl, 1909, S. 490. — Siehe auch Euler, Z. angew. Chem. 35, 580; (1922). — Ebenso dissociiert nach EULER (Lieb. Ann. 330) $NO_2H$ in $NO^+$ und $OH^-$.

[2] E. D. Huges, C. K. Ingold, R. I. Reed Nature 158 448; 1946 C. K. INGOLD et al. Soc. 1938, 929. — 1950, 2400, 2441, 2452, 2467, 2559, 2628, 2657, 2678. — Siehe auch GILLESPIE, E. D. HUGES, C. K. INGOLD, D. J. MILLEN, R. I. REED, Nature, 163 599; 1949.

[3] C. K. INGOLD, Soc. 1950, 2400 u. 2559. Bei diesen Arbeiten geht INGOLD aus von seinen reaktionskinetischen Studien.

[4] M. S. KHARASCH u. WERTHEIMER, J. Am. Chem. Soc. 68. 1871; (1946).

[5] MARQUIS C. r. 132 141 (1901) und 134 777 (1902).

[6] Nebenprodukt Acetyl-nitrofuran, J. Am. Chem. Soc. 53, 1142 (1931).

Bei Wiederholung dieses Versuchs unter genau gleichen Bedingung erhielten wir nur ein gelbes Öl. Auch bei Verwendung von Eisessig und konz. Salpetersäure bei Zimmertemperatur entstand unter Wärmeentwicklung (Wasserkühlung) ein stark gelb gefärbtes Öl, aus dem durch Umkrystallisieren aus Äther gelbbraune Krystalle gewonnen werden, die unter Zersetzung bei 87° schmolzen.

Unter den *aliphatischen* Substraten der Nitrierung[1]) sind hier besonders die *Amide* Guanidin, Harnstoff und Urethan zu nennen, ferner Dicyandiamid und Biuret, wie schon aus den Ergebnissen von Franchimont[2]) und von Thiele[3]) hervorgeht, die mit rauchender Salpetersäure in Gegenwart von konc. Schwefelsäure nitriert haben[4]).

Zum Verlauf der durch Franchimont und Thiele gut bekannt gewordenen Nitrierung der Carbamingruppe[5]) findet man Analogien in der aromatischen Serie und zwar bei Phenol (Blanksma) und bei Anilin (insofern die Salpetersäure bei der Einwirkung auf Anilin intermediär unter Nitramidbildung zuerst in die Aminogruppe eintritt und von dort aus in den Benzolkern wandert[6]). Es handelt sich um eine »indirekte Substitution» im Sinne von Blanksma. Diese für die Kenntnis der Nitrierungs-Zwischenprodukte wichtige Reaktion wird hier weiter studiert.

Aus dem von Lachmann und Thiele erwähnten Acetamid haben wir ein stabiles Nitramid nicht gewinnen können (Chem. Ber. 27, 1519; 1894. — Lieb. Ann. 288, 267; 1895).

## Phosphorylierungs-Versuche

Versuche, die Phosphorylierung des Benzolkernes in analoger Weise durchzuführen wie die Nitrierung, also etwa durch Erhitzen von Benzol mit $HPO_3$ in stark saurer Lösung, sind bekanntlich nicht gelungen. Die Zahl der Valenz-Elektronen von P und N ist allerdings gleich, aber ihre Verteilung auf die Energie-Niveaus ist verschieden. P strebt nicht, wie N, seine Elektronen-Schaale zu ergänzen und gibt keine Elementarladung ab zur Bildung von $PO_2^+$.

Während bei der *Salpetersäure* durch den grossen Proton-Ueberschuss der stark sauren Lösungen die Elektronen-Anteiligkeit, von O zu H also die Bindung zwischen O und H verstärkt, die Bindung $HO^- \ NO_2^+$ dagegen wesentlich gelockert ist (da der Schwerpunkt jedes der beiden bindenden Elektronpaare durch den

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[1] Ueber die Nitrierung ungesättigter aliphatischer Kohlenwasserstoffe siehe H. Wieland (Chem. Ber. 53, 203 (1920).

[2] A. P. N. Franchimont, Rec. Trav. Chim. Pays-Bas 10, 231 (19.

[3] J. Thiele, Lieb. Ann. 270, 1 (1892).

[4] Die Verwendung von Guanidinnitrat und Schwefelsäure zur Nitrierung des Guanidins bedeutet eine Verbesserung der Methodik (vgl. Organic Syntheses 7, 68 (1927). — Ueber die Einwirkung des Nitronium-Ions auf höhere aliphatische Fettsäuren siehe Euler u. H. Hasselquist, dieses Arkiv 26 A Nr 23 (1948). — Euler, H. Hasselquist u. U. Lööv, ebenda A, Nr 37 (1949).

[5] Zur Mesomerie der Säureamide siehe F. Arndt u. B. Eistert, Chem. Ber. 71, 2040 (1930).

[6] E. Bradfield u. K. J. P. Orton, J. Chem. Soc. 1929, 915. — K. J. P. Orton u. C. Pearson, J. Chem. Soc. 93, 725 (1908). — K. J. P. Orton et al. Brit. Assoc. Reps 1912, 117. — Chem. News 106 236 (1912). Auch an Phenol kommt nach Van Alphen indirekte Substitution vor (Rec. Trav. Chim. Pays-Bas 46, 807 (1927). Siehe auch E. Ziegler. (Scientia Pharmac. 19, 10; 1951). — E. Ziegler u. G. Zigeuner, (Mh f. Chem. 80, 359; 1949). Für die Nitrierung von Phenol mit *verdünnter* Salpetersäure (ohne Schwefelsäure) fand Stig Veibel (Chem. Ber. 65; 1932) einen anderen Mechanismus.

Protonüberschuss verschoben ist), so ist dies bei $HPO_3$ offenbar nicht oder in viel geringerem Grade der Fall; aus der *Metaphosphorsäure* lässt sich sogar in Gegenwart sehr hoher Schwefelsäure-Konzentrationen das Anion $PO_2^+$ nicht hervorrufen.[1])

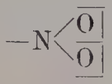
Auch bei gleicher Konzentration der Ionen $NO_2^+$ und $PO_2^+$ könnte der erwähnte reaktionskinetische Unterschied auf der verschiedenen specifischen Reaktionsfähigkeit (Zahl der erfolgreichen Reaktionsstösse) und diese auf dem verschiedenen Grad ihres elektrophilen Charakters beruhen.

Die Einführung von $PO(OH)_2$ bezw. $PO_2$ und $PCl_4$ gelingt bekanntlich auf indirektem Weg[2]):

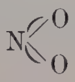
Phosphinobenzol, $C_6H_5PO_2$ entsteht nach MICHAELIS aus Phenylphosphinsäure (= Phosphenylsäure), $C_6H_5PO(OH)_2$ und Phosphenylchlorid, $C_6H_5PCl_2$.

### B. Spaltung aromatischer Nitro-Verbindungen.

Die beiden O-Atome der symmetrisch gebauten Nitrogruppen sind gleichwertig. Dies kann zum Ausdruck gebracht werden durch mesomere Formeln, oder durch das Symbol



oder



Jedenfalls tritt der elektrophile Substituent $NO_2$ als Kation aus dem Molekülverband aus.[3])

Aus dem Mononitrobenzol lässt sich bekanntlich die Nitrogruppe sogar in stark alkalischer Lösung nur schwer abspalten. Die dem unsubstituierten Benzolkern und der Nitrogruppe gemeinsame Elektronenwolke bedingt ein so stabiles und symmetrisches Gebilde, dass der in wässriger Lösung mit der Abspaltung von $NO_2$ verknüpfte Eintritt von OH in den Benzolkern nicht begünstigt wird. Erst in *mehrfach* substituierten Benzolkernen ist die Bindung der Nitrogruppe gelockert.

### Methodisches

Um festzustellen, unter welchen Bedingungen die $NO_2$-Abspaltung aus den verschiedenen Nitroverbindungen erfolgt[4]), haben wir dieselben i wässriger Lösung oder Suspension unter Rückfluss oder in verschlossenem Rohr auf 100° erhitzt und haben in von Zeit zu Zeit herausgenommenen Proben den noch nicht gespaltenen Anteil des Ausgangsmateriales durch Reduktion mit $SnCl_2$ nach LIMPRECHT[5]) quantitativ ermittelt.

$$R.\,NO_2 + 3\,SnCl_2 + 6\,HCl = R.\,NH_2 + 3\,SnCl_4 + 2\,H_2O$$

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[1] Vgl. B. EISTERT, Chemismus und Konstitution I. Bd. (Stuttgart, 1948) S. 30: »Die Tendenz sich onium-artig zu betätigen, ist je nach der Art der vorhandenen und des hinzukommenden Liganden für jedes Zentralatom verschieden.»

[2] A. MICHAELIS, Chem. Ber. 10, 810 (1877). — Lieb. Ann. 181. 265 (1876).

[3] C. K. INGOLD, Soc. 1935, 246.

[4] Quantitative Bestimmungen der $NO_2$-Abspaltung aus Nitrobenzolen lagen noch nicht vor.

[5] LIMPRECHT, Chem. Ber. *11*, 35, (1878).

Die abpipettierten Proben wurden mit Wasser verdünnt und mit Sodalösung bis zur völligen Auflösung des zunächst entstandenen Niederschlages versetzt. Nach weiterem Verdünnen mit Wasser und Zusatz von Stärke wurde das nicht verbrauchte $SnCl_2$ durch Titration mit Jod-Jodkalium bis zur bleibenden Violettrotfärbung gemessen.

Die Festigkeit, mit der die Nitrogruppe am Kern haftet, kommt in folgenden Spaltungsergebnissen zum Ausdruck:

| Substrat | % $NO_2$ abgespalten bei 100° | |
|---|---|---|
| Mononitrobenzol | Theor. max. | 37,4 |
| | Nach 3 Std. 100° 2 n NaOH | 2,0 |
| | Nach 3 Std. 100° 2 n HCl | 0,3 |
| o-Dinitrobenzol | Theor. max. 1 $NO_2$ | 27,4 |
| | Theor. max. 2 $NO_2$ | 54,7 |
| | Nach 1,25 Std. 1 n NaOH | 14,2 |
| | 2 Std. 1 n NaOH | 19,9 |
| | 5 Std. 1 n NaOH | 27,2 |
| o-Nitrophenol | Theor. max. | 33,1 |
| | Nach 4 Std. 1 n NaOH | 2,9 |

Diese Substitution der einen $NO_2$-Gruppe durch OH verläuft, wie Fig. 1 zeigt, regelmässig und folgt der Formel für monomolekulare Reaktionen, ($K = 0{,}26$) obwohl anzunehmen ist, dass der Vorgang mit einer Lockerung der $NO_2$-Gruppe und Bildung eines Reaktionsknäuels (W. KOSSEL[2]), M. TRAUTZ) beginnt. Bei der Abtrennung verlässt die Nitrogruppe den Benzolkern mit einer positiven Ladung unter Bildung salpetriger Säure, was mit der bekannten, von uns nachgeprüften hohen Stabilität des Mononitrophenols (siehe Tabelle) in Übereinstimmung steht. Die entsprechende Abspaltung aus o-Nitro-amino-benzol konnte L. SIHLBOM 1950/51 durch die von ihm entdeckte intramolekulare Diazotierung nachweisen.

## C. Spaltung aliphatischer Nitro-Verbindungen

100 mg *Nitro-Urethan* (dargestellt nach THIELE und LACHMANN (Ann. 288, 287; 1895), farblose Tafeln, Schmpt. 63—64°, wurden bei Zimmertemperatur mit 5 ml 2 n NaOH versetzt; sofort trat Gasentwicklung ein (dagegen konnte kein Ammoniak-Geruch festgestellt werden).

Ein kleiner Teil der Lösung A wurde mit Jod-Jodkalium-Lösung versetzt, wodurch Jodoform-Geruch auftrat. Als der Hauptteil der Lösung A nach Ab-

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[1] LOBRY DE BRUYN (Rec. 2, 236; 1883) erhielt beim Erhitzen von o-Dinitrobenzol mit alkoholischer Kaliummethylat-Lösung o-Nitro-anisol. Es darf in diesem Zusammenhang vielleicht betont werden, dass die hydrolytische »Abspaltung» der Nitrogruppe eigentlich eine Substitution ist, da die Nitrogruppe in wässriger saurer oder alkalischer Lösung durch OH ersetzt wird. Der saure Charakter des Dinitrobenzols lässt sich in wässriger oder wässrig-alkoholischer Lösung elektrometrisch deutlich nachweisen.

[2] W. KOSSEL, Ann. d. Phys. 49 229; 1916. — Z. f. Physik 395; 1920. — Siehe auch M. TRAUTZ, Lehrb. d. Chemie, W. DE GRUYTER, 1924. Ein in Dissociation befindliches Molekül wird während der Dissociation — etwa als Radikal — von der 2. Reaktionskomponente getroffen.

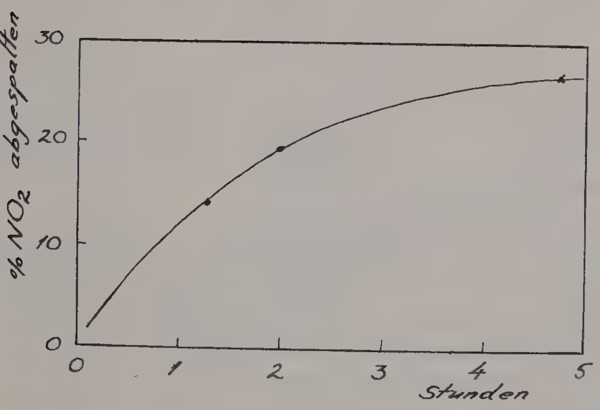

Fig. 1.

schluss der Gasentwicklung neutralisiert wurde, setzte die Bildung von $CO_2$ ein. Wurde die essigsaure Lösung A mit überschüssigem Silbernitrat versetzt, so krystallisierte sofort ein Silbersalz aus, das sich durch sein Verhalten als $Ag_2N_2O_2$ erwies. Die Bildung von Distickstoffoxyd kann also formuliert werden:

$$C_2H_5OOC\text{—}NH\text{—}NO_2 \rightarrow N_2O + CO_2 + C_2H_5OH$$

220 mg *Nitro-Guanidin* wurden mit 5 ml 2 n NaOH auf 60° erwärmt, wobei $NH_3$ sofort abgespalten wurde. Dagegen erhielten wir keine $N_2O$-Abspaltung (vgl. Thiele Ann. 270 18; 1892). Nach vollendeter $NH_3$-Entwicklung wurde die alkalische Lösung mit Essigsäure angesäuert und mit Ueberschuss von Silbernitrat versetzt. (Beim Ansäuern entstand lebhafte $CO_2$-Entwicklung.) Beim Zusatz von Silbernitrat fiel ein schön krystallisierendes Silbersalz aus, das beim Umkrystallisieren aus Wasser in grossen Stäbchen vom Schmpt. über 290° erhalten wurde. Dasselbe zersetzte sich bei Einführung in die Flamme explosionsartig; es erwies sich identisch mit Silberhyponitrit, $Ag_2N_2O_2$.

Auch *Nitro-Harnstoff* (500 mg) zeigte nach Zugabe von 10 ml 2 n NaOH bei 80° das gleiche Verhalten wie Nitro-Urethan und Nitro-Guanidin. Nach Zusatz von Silbernitrat krystallisierte Silberhyponitrit aus. Wir fanden, dass Nitro-Harnstoff in alkalischer Lösung gespalten wird in $NH_3$, $CO_2$ und $N_2O$.

Nach Messungen von E. Baur (Zs. physik. Chem. 23, 409; 1897) sind die genannten Nitramide stärkere Säuren als Essigsäure; ihre Diss.-konst. K sind:

$$\text{Nitroharnstoff } K = 5.10^{-5} \qquad \text{Nitrourethan } K = 5.2.10^{-4}.$$

Ihre Salze mit 1 Äquivalent NaOH sind also kaum merkbar hydrolysiert (praktisch neutral) (Für Nitroguanidin ist von J. K. Wood eine sehr kleine basische Diss. Konstante, $K = 10^{-15}$ (40°), angegeben worden). Bei diesen Nitramiden wird man als erste Stufe der Spaltung eine Hydrolyse annehmen, durch welche die Nitramidgruppe bei oder nach der Umwandlung in NaON. NOH abgetrennt wird.[1]

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[1] Über Hantzschs irrtümliche Formulierung primärer Nitramine vgl. Euler, Chem. Ber. 39, 1607. — Siehe auch A. H. Lamberton, Quart. Rev. 5, 75; 1951.

## D. Der Verlauf der Spaltung des Nitramids

Das Endprodukt der alkalischen Spaltung des Nitramids ist $N_2O$. Thiele nimmt als erste Phase der Reaktion die Bildung von Imidosalpetersäure an. Bei der Neutralisation des Nitramids, das als schwache Säure, Diss. Konst. $K = 3.10^{-7}$, fungiert, tritt eine Umlagerung in die Form HON. NOH ein, für welche man eine erste Dissociationskonstante $K_1 = 2.10^{-7}$ und eine zweite Diss. Konstante $K_{II} = \sim 10^{-12}$ annehmen kann.

Die Geschwindigkeit der Nitramid-Spaltung *in saurer wässsriger* Lösung ist relativ gering (siehe Fig. 2 u. 3). In *alkalischer* Lösung steigt die Geschwindigkeit der $N_2O$-Entwicklung mit dem Grad der Salzbildung des Substrates, und zwar weniger mit der Konzentration der Ionen $^{-}$ON. NOH (Fig. 3, Kurve II), sehr stark hingegen mit der Konzentration der Ionen $^{-}$ON. NO$^{-}$, deren specifische Zerfalls-Geschwindigkeit durch die Aufnahme der zweiten Ladung wesentlich vergrössert wird.

Zur pH-Abhängigkeit der Nitramid-Spaltung[1]) mag hier folgendes bemerkt werden:

In Bezug auf die Theorie der reaktionsvermittelnden Ionen soll zunächst an den Grundsatz erinnert werden, dass die Reaktionsfähigkeit des Substrates stark durch die Aufnahme einer Ladung gesteigert wird. Bei Basen- und Säure-Katalysen sind es in erster Linie die *durch die Salzbildung Substrat-Katalysator entstehenden Substrat-Ionen*, welche die Hydrolyse vermitteln.[2])

Wie aber schon früher[3]) betont wurde, darf man hierbei nicht jede Ionenbildung an einem Substrat als reaktionsvermittelnd in Rechnung setzen; vielmehr kann nur diejenige Ionisierung in Betracht kommen, welche wesentliche Elektronen-Verschiebungen in der reagierenden Gruppe des Substrates veranlasst. Wir haben früher im Sinne von C. Fajans auf die durch die Ionisierung des Substrates eintretenden *Deformationen* hingewiesen, bei welchen wohl meist »Entkoppelungen» stattfinden.[4])

Die experimentellen Daten für die Berechnung der Nitramid-Spaltung auf Grund der Theorie der reaktionsvermittelnden Ionen sind noch nicht abschliessend ermittelt. Einstweilen soll nur hervorgehoben werden, dass das Nitramid als Säure durch die elektrolytische Dissociationskonstante $K = 2—3.\ 10^{-7}$ (die vielleicht noch einer kleinen Korrektion bedarf), charakterisiert ist, während die Dissociationskonstante als Base so klein ist, dass sie sich bisher einer genauen Messung entzogen hat.

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[1] Durch den Zusatz von Salzen schwacher organischer Säuren wird die $N_2O$-Abspaltung um so stärker katalysiert, je kleiner die Diss. Konst. dieser Säuren sind, wie auch aus einer Tabelle von Brønsted u. K. Pedersen (Zs. physik. Chem. 108, S. 198) hervorgeht. Brønsted hat mit Mitarbeitern zahlreiche weitere Versuche über die Nitramid-Spaltung angestellt (Z. physik. Chem. 117, 289; 1925. — Ebenda 169, 361 u. 379; 1934) und hat dieselben mit einer reaktionskinetischen Theorie versehen, auf die wir hier nicht eingehen wollen.

[2] Euler, Zs. physikal. Chem. 36, 66; 1901. — Rapport du II. Conseil de chimie de l'Institut Intern. de Chimie Solvay 1925. — Euler u. Rudberg, Zs. anorg. u. allg. Chem. n37, 244; 1923. — Euler u. A. Ölander, Zs. physikal. Chem. 131, 107; 1927.

[3] Euler, Zs. physikal. Chem. 47, 353; 1904.

[4] Wenn wir annehmen, dass die specifische Reaktionsfähigkeit der *Substrat-Ionen* sehr viel grösser ist als die des nicht dissociierten Substrates wird damit keineswegs behauptet, dass die Reaktionsfähigkeit des freien Substrates gleich Null ist. Auch können sehr wohl die Substrate mit anderen Stoffen als Säuren und Basen, z. B. mit Salzen, Verbindungen von hoher specifischer Reaktionsfähigkeit bilden. Siehe hierzu auch H. Meerwein (Lieb. Ann. 455, 227; 1927) Ueber die Vergrösserung der Ionisationsfähigkeit schwacher Elektrolyte durch Komplexbildung.

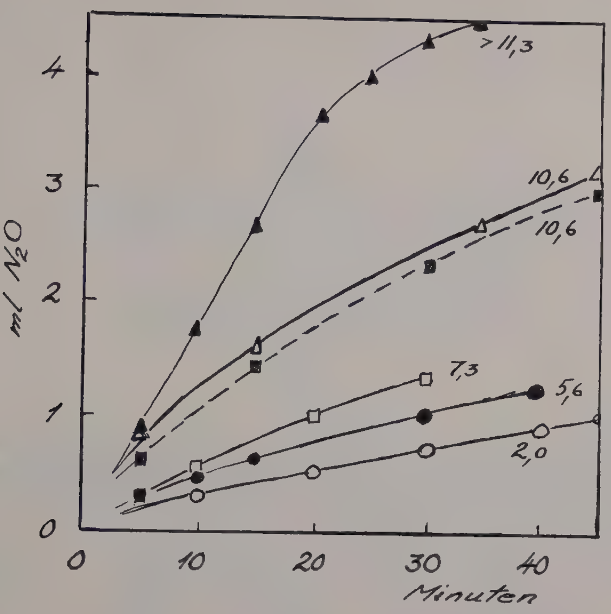

Fig. 2.

Messbare Salzbildung tritt am Nitramid fast nur mit starken Basen, kaum mit starken Säuren ein. Die pH-Abhängigkeit der Nitramid-Spaltung geht aus den Kurven der Fig. 2 hervor.

Zunahme der Spaltungsgeschwindigkeit mit der Konzentration der katalysierenden *Base*, aber ganz geringe Spaltung in saurer Lösung. (Ob diese letztere den undissociierten Nitramid-Molekülen oder einer anderen Molekülart zukommt ist noch nicht entschieden).

Der Verlauf der Reaktionsgeschwindigkeit - pH - Kurve zwischen pH 9 und 12 deutet darauf hin, dass dem doppelt geladenen Nitramid-Ion eine hohe specifische Zerfallsgeschwindigheit zukommt (Fig. 2 u. 3).

Die Form der Reaktionsgeschwindigkeit - pH - Kurve des Nitramids (Spaltung fast nur auf der alkalischen Seite ist analog der für Benzolsulfosäure-Ester festgestellten; diese Ester werden bekanntlich nur durch Alkali, nicht durch Säuren hydrolysiert [1]). Durch ihr Verhalten zu alkalischen und sauren Katalysatoren unterscheiden sich die Carbonsäure-Ester wesentlich von den Sulfosäure-Estern. Für diese beiden Ester-Typen hat K. G. KARLSSON die pH-Abhängigkeit, und — wie auch IVAN BOLIN [2]) — für die Carbonsäure-Ester die Stabilitätsmaxima festgestellt, die im allgemeinen zwischen pH 4 und 5,5 liegen.

Was nun die specifische Reaktionsgeschwindigkeit der durch Alkali entstehenden Ester-Salze bezw. Ester-Ionen betrifft, so sei hier auf die Darstellung von B. EISTERT [3]) verwiesen, auf die wir in einer späteren Mitteilung noch zurückkommen.

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[1] Vgl. K. G. KARLSSON, Zs. anorg. u. allg. Chem. 145, 1; 1925; Gradual-Abhandlung Stockholm. — Siehe auch WEGSCHEIDER u. PRAETORIUS, Mh. f. Chem. 26, 1; 1907. — PRAETORIUS, Mh. f. Chem. 26, 1; 1905. — SKRABAL u. Mitarb. Mh. f. Chem. 45, 141; 1926.

[2] I. BOLIN, Zs. f. anorg. u. allg. Chem. 143, 201; 1925.

[3] B. EISTERT, Tautomerie u. Mesomerie, Stuttgart, Enke, 1938.

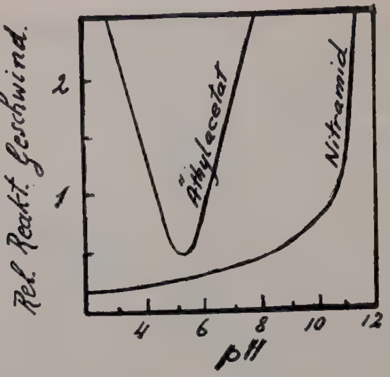
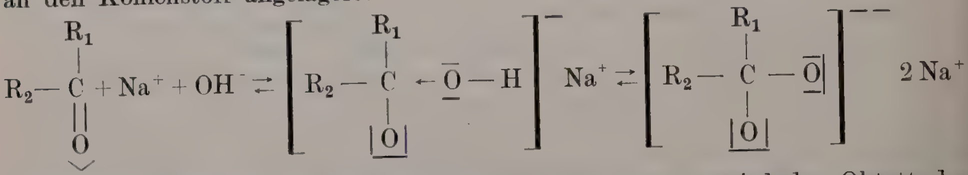


Fig. 3.

”Bei der Einwirkung von Alkalilauge auf Carbonylverbindungen wird primär OH-Ion (bzw., da dessen Proton von überschüssiger Lauge abgelöst wird, $O^{--}$-Ion) an den Kohlenstoff angelagert:

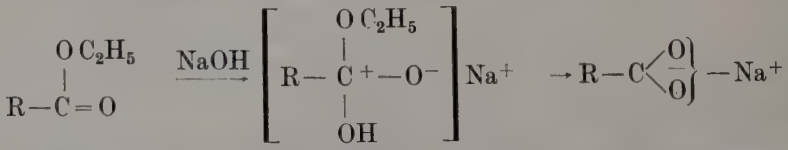


$$R_2-\overset{\displaystyle R_1}{\underset{\displaystyle \overset{\|}{O}}{C}} + Na^+ + OH^- \rightleftarrows \left[R_2-\overset{\displaystyle R_1}{\underset{\displaystyle |\underline{O}|}{C}} \leftarrow \overline{\underline{O}}-H\right]^- Na^+ \rightleftarrows \left[R_2-\overset{\displaystyle R_1}{\underset{\displaystyle |\underline{O}|}{C}} - \overline{\underline{O}}|\right]^{--} 2\,Na^+$$

Durch die Einlagerung des weiteren el-affinen Atoms wird das Oktett des Carbonyl-C-Atoms *noch instabiler;* das hat zur Folge, das einer der Substituenten R, und zwar der El.-affinere, als *Anion* (mit El.-Paar) ablösbar wird. Ist $R_1$ = Alkoxyl, so entspricht dies der alkalischen Verseifung der *Ester.*“

In Fig. 3 ist durch eine Kurve für Äthylacetat die Abhängigkeit der Spaltungsgeschwindigkeit von pH mit derjeningen für Nitramid zusammengestellt; letztere entspricht, wie erwähnt, derjenigen der Sulfosäure-Ester.

Während die Sulfosäure-Ester ausgesprochen sauren Charakter besitzen und keine oder äusserst geringe basische Eigenschaften zeigen, sind die Carbonsäure-Ester zwar ebenfalls überwiegend sauer, aber die Carbonsäure-Ester bilden auch, wie die Äther, Verbindungen mit starken Säuren, und demgemäss entstehen durch Aufnahme eines Protons Kationen. — Wir haben die Salzbildung mit NaOH und die Verseifung folgendermaassen formuliert.



$$R-\overset{\displaystyle O\,C_2H_5}{\overset{|}{C}}=O \xrightarrow{NaOH} \left[R-\overset{\displaystyle O\,C_2H_5}{\underset{\displaystyle OH}{C^{+}}}-O^-\right] Na^+ \rightarrow R-C\left\langle\begin{matrix}O\\O\end{matrix}\right\}-Na^+$$

Die Aufnahme der elektrischen Ladung veranlasst die Entkoppelung der Elektronen zwischen dem Säure- und dem Alkohol-Radikal, durch welche die Hydrolyse zustande kommt.

Stockholm, Universität, Vitamin-Institut und Institut für organ.-chemische Forschung.

Tryckt den 11 februari 1952

Uppsala 1952. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 61

Communicated 24 October 1951 by A. Westgren and Karl Myrbäck

# On the structure of phosphors of the aluminium oxide-calcium fluoride type activated with manganese

By Gunnar Günther and Harald Perlitz

With 3 figures in the text

The study of a new type of phosphors, aluminium oxides activated with manganese and modified with fluorides of alkaline-earth metals, revealed the presence of a new phase in the $Al_2O_3$–$CaF_2$ system. This structure corresponded to the base composition of the phosphor with the formula $5\ Al_2O_3 \cdot CaF_2$ (1). This was of particular interest owing to the approximate parallelism between the development of this phase and its luminescent properties under excitation by long-wave ultra-violet rays (3650 Å). As a more detailed knowledge of the phase diagram of the system is important for an interpretation of the luminescent manifestations of this type of phosphors — even with other activators than manganese — a considerable number of phosphors differing in composition and prepared at different temperatures have been examined by X-rays. Since the preparation of the phosphors at first hand required an oxidizing atmosphere, the top temperature range had to be limited to 1 450° C.

## Methodical

For ways of preparation and excitation of the phosphors, as well as for measuring of their luminescence, reference is made to a previous paper of Günther et alii (1). After the temperature in the stove which contained the properly mixed ingredients had been raised from room temperature to the desired maximum temperature, which was achieved in 3 hours, the ingredients were baked for two additional hours at the maximum temperature. This proved fully sufficient for the achievement of optimal luminescence with comparatively small specimen quantities. It should be born in mind in this connection that in finished phosphors, as a rule, the components neither are present in strictly stoichiometric proportions, nor have their mutual reactions generally proceeded until a definite state of equilibrium. Accordingly the primary aim of this investigation was to elucidate the variations in luminescence and to find a relation between these variations and any intermediary phases in the base material.

All diffraction patterns were taken in Debye-Scherrer powder cameras with unfiltered Cr-*K*-radiation, the specimens being rotated.

## Experimental

In Pascal's phase diagram for the $CaF_2$–$Al_2O_3$ system no intermediate phases occur (2). According to our X-ray examinations, however, with the ($x\ Al_2O_3 : CaF_2$):

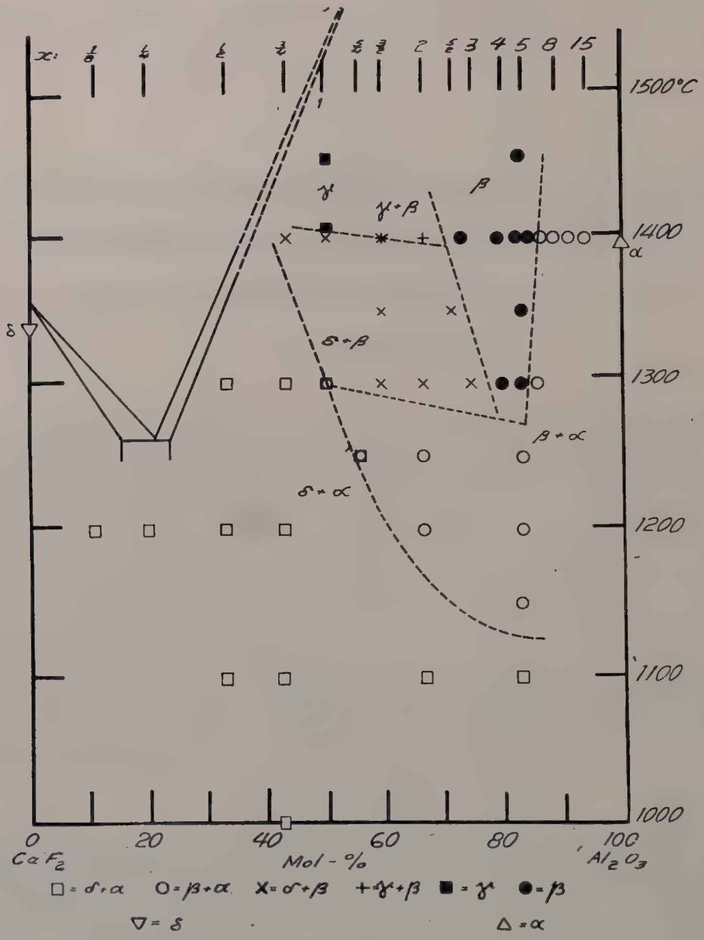


Fig. 1. Phase diagram of ($x\,Al_2O_3$: $CaF_2$) : Mn (0.045) baked in air.

Mn(0.045) phosphors, two such phases occur at temperatures higher than 1100° C. These are shown in figure 1 in the phase diagram, where $\alpha$ denotes the $Al_2O_3$-phase, $\beta$ the $5\,Al_2O_3 \cdot CaF_2$-phase, $\gamma$ the $Al_2O_3 \cdot CaF_2$-phase and $\delta$ the $CaF_2$-phase. The $\beta$-phase, an intermediate phase with a rather complicated diffraction pattern, is the bearer of the luminescence. It is homogeneous within a wedge-shaped region with the edge somewhere between the isotherms 1300° C and 1250° C. Although an extension of the final baking time at 1200° C from 2 to 6 hours or more will advance the reaction $5\,Al_2O_3 + CaF_2 \rightarrow 5\,Al_2O_3 \cdot CaF_2$ nearer to completion, this does not always result in an intensified luminescence, which is known to depend not only on the macrostructure, but also — and as least as much — on several other factors, such as grain growth, the structural incorporation of the activator into the lattice and the formation of other internal lattice disturbance. Above 1400° C the $\gamma$-phase appears. No photo-luminescence whatever is associated with the $\gamma$-phase as base

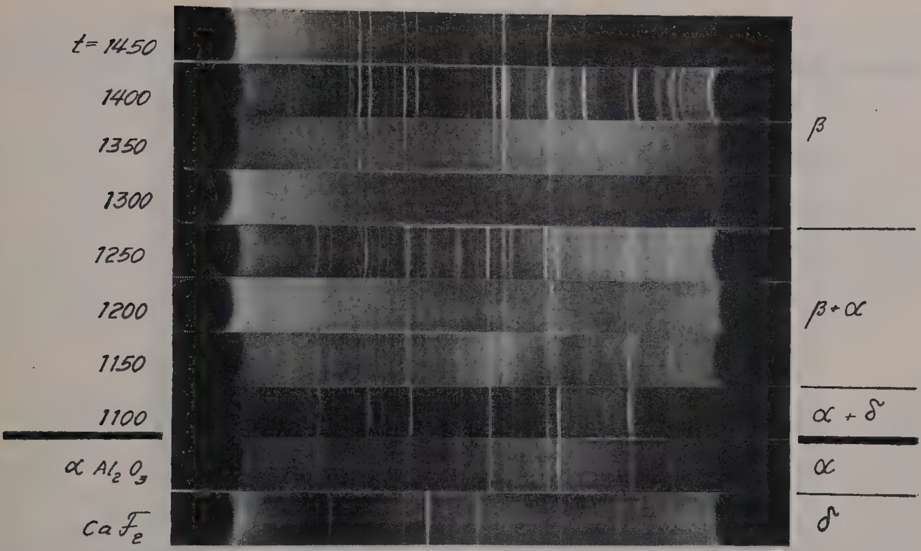


Fig. 2. Powder patterns of 5 $Al_2O_3 \cdot CaF_2$ : Mn (0.045) baked in air at varying temperatures.

and manganese as activator, and owing to the increased proportion of fluoride — 1 mole $CaF_2$ per mole of $Al_2O_3$ — the specimens become hard-baked by the heating, which in general is unfavourable for the manufacture of phosphors. Figure 2 shows powder patterns of 5 $Al_2O_3 \cdot CaF_2$:Mn(0.045) obtained from specimens baked at different temperatures, and figure 3 those of ($x$ $Al_2O_3$:$CaF_2$):Mn(0.045) all baked at 1400° C.

Because the range of the $\beta$-phase is so restricted, the manufacturing of good phosphors with this pure structure as base requires particular care with regard to the homogeneity of the component mixtures to be baked, as well as to the reaction tendencies of the individual components. This is intimately related to the surface area and the surface conditions of the individual grains. Ammonium-aluminium-sulphate, aluminium nitrate, aluminium hydroxide and others, when heated, give all aluminium oxide, which in being baked together with fluor-spar leads more or less promptly to the formation of the $\beta$-phase, independently of the presence or absence of the trifling amount of manganese. The intensity of the luminescence, however, will depend on the origin of the base materials used, even if they are of the same degree of purity. Local over-concentrations of fluor-spar in the baking mixture are liable to cause non-luminescent dots in the baked mass. All test series so far carried through seem to indicate that oxide from ammonium-aluminium-sulphate, baked in the most cautious way, is most active and admits of comparatively short baking times. If the luminescence is used as an indicator and the baking times are properly adapted, it is possible to a rather large extent to estimate the kinetics of the solid state reaction between calcium fluoride and aluminium oxides with different prehistory. This is a quick way of examination.

Regarding the question whether fluorescence is obtained from aluminium oxides modified with fluoride if other activators than manganese are used, a glance at the phase diagram will show that it is suitable to examine specimens lying on the vertical

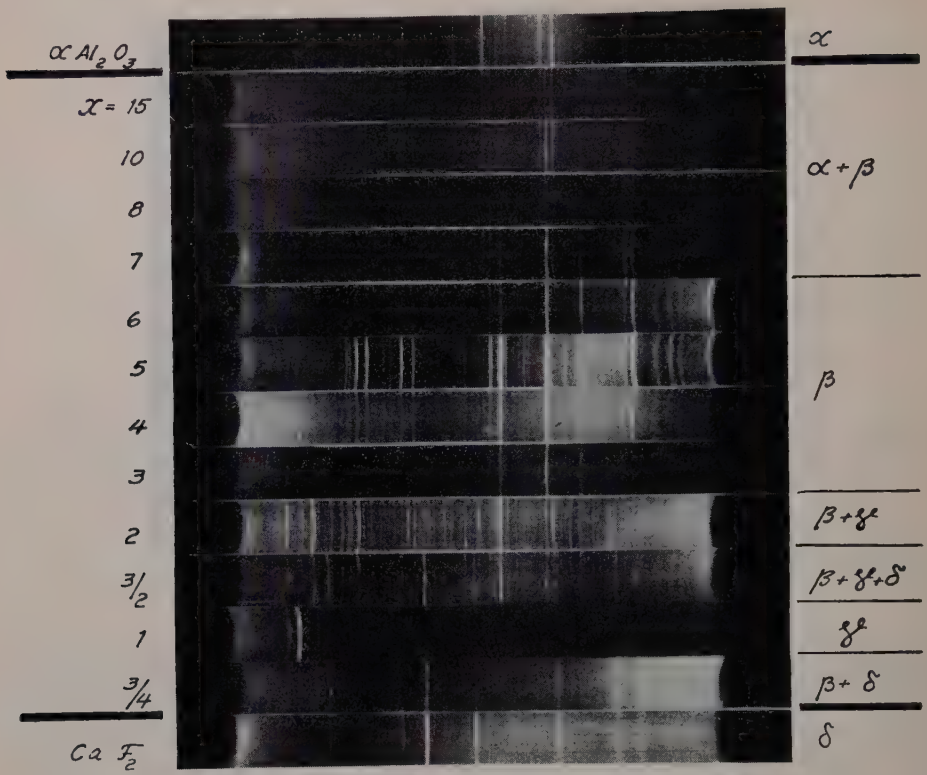


Fig. 3. Powder patterns of ($x$ $Al_2O_3$ : $CaF_2$) : Mn (0.045) baked in air at 1400° C.

$x=5$ and those on the isotherm 1425° C for $x$-values between $\infty$ and 3/4. The examination will then cover specimens which are certainly interesting. Though in the case of manganese the $\beta$-phase is associated with luminescence, this is not always the case with other activators. Thus, for example, a yellow or green photo-luminescence results after baking at 1000° C specimens of the composition $Al_2O_3:CaF_2 = 5:1$ activated with vanadine or uranium, in which the $\beta$-structure is absent. A detailed investigation on the effects of different activators is going on which may be expected to give more reliable data on the ways of the structural incorporation of atoms of the activator into the respective space lattices.

A short after-baking in dry hydrogen of the produced manganese-activated phosphors will give a luminescence covering a rather broad range in the visible if excited with short-wave ultra-violet radiation (2537 Å), the strong red luminescence excited by 3650 Å practically having disappeared (3). Corresponding powder-patterns, however, showed no structural changes.

If the above-mentioned phosphors had been prepared by direct baking of the components in dry hydrogen, no useful photo-luminescence was obtained. Specimens of the composition 5 $Al_2O_3 \cdot CaF_2$ baked in hydrogen at 1100°, 1250° and 1400°, respectively, for 30 minutes to two hours, contained mostly the $\alpha$-phase and gave no photo-luminescence. Thus, the development of the different phases largely depends on the surrounding gas atmosphere. This has long been known in the practical manufacture of phosphors. Among other things, the velocity of the solid state reactions, or the activator's valency, is influenced. In our case, in order to obtain a 5 $Al_2O_3 \cdot CaF_2$:Mn(0.045) luminophor with luminescence if excited by 2537 Å, it is necessary firstly to build up the $\beta$-phase in a comparatively short time by baking, for instance, in air, and secondly to undertake an after-baking in hydrogen to transform into divalent manganese ions those of higher valency corresponding to the luminescence excited by 3650 Å. To ensure the most uniform distribution of the manganese, it should be added previously to the baking of the components to the $\beta$-phase. That gives better results than adding the manganese to the $\beta$-phase at the reducing after-baking.

## SUMMARY

As a result of X-ray examination a phase diagram is established for phosphors consisting of aluminium oxide and calcium fluoride baked in air. Two intermediate phases have been met with. Only the intermediate phase poorer in fluoride shows a fluorescence if manganese is the activator and if excited by 2537 Å and 3650 Å, the manganese in the former case being bivalent, in the latter case of higher valency.

The Research Laboratory of Lumalampan AB, Stockholm.

REFERENCES. (1) **Günther** et alii, Arkiv Kemi Bd 1, Nr 64 (1950). — (2) **Pascal, P.,** Elektrochem. 19 (1914), 611. — J. Am. Ceram. Soc. Phase Diagrams for Ceramists, Nov. 1, 1947, Part II, 123. — (3) **Günther, G.** and **Andersson, G.,** Swedish patent 133519.

Tryckt den 26 februari 1952

Uppsala 1952. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 3 nr 62

Mitgeteilt am 10. Oktober 1951 durch H. v. EULER und KARL MYRBÄCK

# Eine elektrometrische und polarographische Untersuchung über Komplexbildungen zwischen Borsäure und Polyalkoholen

Von KURT TORSSELL

Mit 7 Figuren im Text

Schon früh hatte man herausgefunden, dass Polyalkohole das Leitungsvermögen der Borsäurelösung erhöhen (1). Dies wurde einer Komplexbildung zwischen der Borsäure und den Polyalkoholen zugeschrieben. Später untersuchten viele Forscher, vor allem BÖESEKEN und seine Schule, diese Komplexe und zeigten, dass es sich um eine Anlagerung von einem oder zwei Polyalkoholmolekülen pro Borsäuremolekül handelte.

Diese Verbindungen sind stärkere Säuren, als es die Borsäure selbst ist. Aus diesem Grunde tritt eine Steigerung der $[H^+]$ ein, und damit ist eine Erhöhung des Leitungsvermögens verbunden (2). Hauptsächlich bildet sich Didiolborsäure, z. B. aus Mannit oder Fruktose und Borsäure. Der Verfasser beabsichtigt in der vorliegenden Untersuchung die Anwesenheit der Monodiolborsäure nachzuweisen. Ferner sollen die Komplexkonstanten der beiden Komplexe errechnet werden, sowie die Möglichkeit geprüft werden, $K_s$ für $HBD_2$ zu berechnen. Das polarographische Verhalten der Fruktose und ihrer Boratkomplexe soll schliesslich untersucht werden.

Es stellen sich folgende Gleichgewichte ein:

I $B^- + D \rightleftharpoons BD^-$
II $B^- + 2D \rightleftharpoons BD_2^-$
III $HB \rightleftharpoons H^+ + B^-$
IV $HBD_2 \rightleftharpoons H^+ + BD_2^-$
V $HBD \rightleftharpoons H^+ + BD^-$
(HB = Borsäure, D = Polyalkohol).

Ausserdem ist denkbar, dass Komplexe vorkommen, die 2 HB pro D enthalten. Die Borsäure ist selbstkomplexbildend, jedoch kann man bei den hier vorkommenden minimalen Borsäurekonzentrationen $(5 \cdot 10^{-3}\text{–}5 \cdot 10^{-2})$ davon absehen.

Aus den Gleichgewichten ergeben sich folgende Gleichungen:

$$1)\quad \frac{[BD^-]}{[B^-]\,[D]} = K_1$$

$$2)\quad \frac{[BD_2^-]}{[B^-]\,[D^2]} = K_2$$

3) $$\frac{[H^+]\,[B^-]}{[HB]} = K_{HB}$$

4) $$\frac{[H^+]\,[BD_2^-]}{[HBD_2]} = K_{HBD_2}$$

5) $$\frac{[H^+]\,[BD^-]}{[HBD]} = K_{HBD}$$

6) $$[HB_{tot}] = [HB] + [B^-] + [BD^-] + [BD_2^-] + [HBD] + [HBD_2]$$

7) $$[D_{tot}] = [D] + [BD^-] + [HBD] + 2[BD_2^-] + 2[HBD_2]$$

8) $$[H^+] + [a] = [B^-] + [BD_2^-] + [OH^-]$$

In der angewendeten Borsäure war eine geringe Alkalimenge, $[a]$, enthalten, siehe unten.

SCHÄFER (3) zeigte, dass $K_{HBD_2}$ grösser als $10^{-4}$ ist und wahrscheinlich auch grösser als $10^{-3}$. Eine Vorstellung über die Grösse des $K_{HBD}$ gewinnt man durch folgende Überlegung.

In einer Lösung, wo $[HB] \sim 10^{-2}$ C und $[D] \sim 1$ C, ist $[H^+] \sim 10^{-3}$ C. $[BD^-]$ lässt sich aus 1) errechnen. $[HBD]$ kann höchstens den Wert $[HB]$ erreichen. Wenn man diese Werte in 5) einsetzt, ergibt sich, dass $K_{HBD} > 10^{-6}$ sein muss, wahrscheinlich um mehrere 10-er Potenzen. Liegt das pH zwischen 4 und 5, so ist die Bildung von HBD, sowie von $HBD_2$ ohne Bedeutung.

Die Gleichungen lassen sich dann folgendermassen vereinfachen:

6 a) $$[HB_{tot}] = [HB] + [B^-] + [BD^-] + [BD_2^-] = [HB] + [H^+] + [a]$$

7 a) $$[D_{tot}] = [D] + [BD^-] + 2\,[BD_2^-] \approx [D] + 2\,[H^+] + 2\,[a]$$

Hieraus lassen sich $[HB]$ und $[D]$ berechnen.

Aus 1), 2), 3), und 8) ergibt sich:

9) $$\frac{[H^+]\;([H^+] + [a]) - K_{HB} \cdot [HB]}{K_{HB} \cdot [HB] \cdot [D]} = K_1 + K_2 \cdot [D]$$

Trägt man das linke Glied der Formel (= A) auf der Y-Achse ab und $[D]$ auf der X-Achse, so erhält man eine Gerade, deren Neigung $K_2$ darstellt. Der Schnittpunkt mit der Y-Achse stellt $K_1$ dar.

## Emk-Messungen

### Experimenteller Teil

Apparatur: Potentiometer, von Radiometer, Kopenhagen. In einem Wasserthermostaten wird die Temperatur bei 25° C ± 0,1° gehalten.

Chemikalien: Borsäure p. a. Baker, einmal aus Wasser umkristallisiert und über $H_2SO_4$ getrocknet; NaCl p. a. Merck, Darmstadt; d-Fruktose C. P. Special, Pfanstiel Chemical Co., Waukegan, USA; d-Mannit C. P. Special, Pfanstiel Chemical Co., Waukegan, USA; Wasser mit $CO_2$-Schutz destilliert.

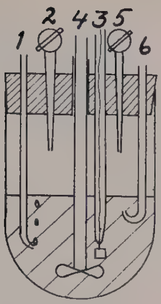


Fig. 1. 1. $N_2$-Zufuhr. 2. Bürette für die D-Lösung. 3. Pt-Elektrode. 4. Rührer. 5. Bürette für NaOH. 6. Leitung zur Kalomelelektrode.

Um die Konstanten in 9) berechnen zu können, ist es notwendig $[H^+]$ zu kennen. $[H^+]$ erhält man durch Messung der Emk der folgenden Kette:

$$+\,\mathrm{Pt} \,/\, \begin{array}{l} QH_2,\ Q \\ 0{,}1\ \mathrm{C\ NaCl} \\ 20\ \mathrm{ml\ HB};\ \mathrm{v\ ml\ D} \end{array} \,/\, 0{,}1\ \mathrm{C\ NaCl} \,/\, 0{,}1\ \mathrm{C\ NaCl},\ Hg_2Cl_2 \,/\, \mathrm{Hg} -$$

($QH_2$, Q bedeutet Chinhydron).

E° wird durch Messung der Emk bei einer bekannten $[H^+]$ bestimmt. $[H^+]$ erhält man aus der Gleichung:

$$-\log [H^+] = \frac{E - E^\circ}{59{,}16}$$

E = Emk in der Lösung.

Sämtliche Messungen wurden in 0,1 C NaCl vorgenommen, und die erhaltenen Gleichgewichtkonstanten gelten somit bei dieser Ionenstärke. Durch die Füllung der Kalomelelektrode mit 0,1 C NaCl ist das Flüssigkeitpotential auf ein Minimum herabgedrückt worden. $K_{HB}$ ist durch Messung der $[H^+]$ einer Boraxlösung bei derselben Ionenstärke bestimmt worden. $K_{HB} = 1{,}15 \cdot 10^{-9}$.

Nach BÖESEKEN und VAN ROSSEM (4) hat das Chinhydron keinen Einfluss auf das Leitungsvermögen der Borsäure.

Um $CO_2$ aus dem Titrationsgefäss (siehe Fig. 1.) ausschliessen zu können, wurde $N_2$ langsam durch die Lösung geleitet. $N_2$ wurde zuvor von eventuellen sauren und basischen Bestandteilen mit NaOH-Lösung und $H_2SO_4$ befreit.

20 ml HB werden mit der D-Lösung, worin auch HB enthalten ist, um [HB] nicht zu verändern, titriert. Nach jedem Zusatz von D misst man die Emk. [HB] wurde zwischen $5 \cdot 10^{-3}$ und $5 \cdot 10^{-2}$ C, und [D] zwischen 0 und $5 \cdot 10^{-1}$ C variiert.

Oben wurde erwähnt, dass die angewendete Borsäure eine geringe Alkalimenge enthält. Diese lässt sich auf folgende Weise bestimmen. $[H^+]$ wird in der reinen HB-Lösung gemessen. Da $K_{HB}$ bekannt ist, kann man $[B^-]$ berechnen, welche in Abwesenheit von Alkali mit $[H^+]$ gleich sein musste. Die Differenz $[B^-] - [H^+]$ ist $= [a]$.

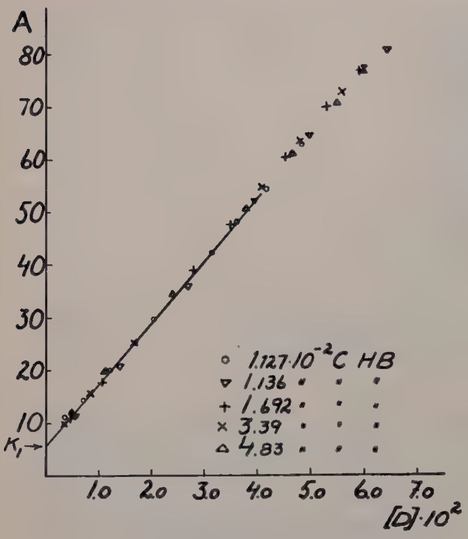


Fig. 2. Mannit, kleine Konzentrationen.

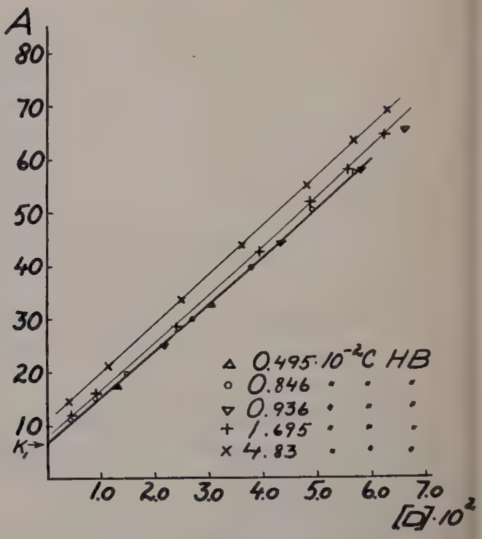


Fig. 3. Fruktose, kleine Konzentrationen.

## Besprechung

Bei niedriger [D] zeigt sowohl die Fruktose- als auch die Mannitkurve einen geradlinigen Verlauf. Jedoch erkennt man bei erhöhter [D] eine schwache Biegung nach oben, und zwar stärker bei Fruktose als bei Mannit. Dies deutet auf die Bildung eines Komplexes mit drei Liganden, $BD_3^-$ hin, die Ursache kann aber auch auf einer Veränderung des Ionenmediums infolge erhöhter [D] beruhen. Die Mannitkurve verändert ihre Lage bei verschiedener [HB] nicht. Dies ist ein Beweis dafür, dass sich nicht mehrkernige Komplexe bilden (5). Daher können keine messbaren Mengen eines Komplexes mit z. B. 2 HB pro D vorhanden sein. Die Fruktosekurve wird indessen bei erhöhter [HB] etwas nach aufwärts verschoben, was also auf eine Bildung von mehrkernigen Komplexen zurückzuführen ist. Bei abnehmender [HB] nähern sich die Kurven einem Grenzwert. Für Mannit hat sich durch graphische Extrapolation $K_1 = 5{,}6 \cdot 10^2$ ergeben. $K_2 = 1{,}16 \cdot 10^5$. Entsprechende Werte für Fruktose sind $K_1 = 6{,}9 \cdot 10^2$ und $K_2 = 0{,}83 \cdot 10^5$. DEUTSCH und OSOLIN (6) geben für Mannit $K_1 = 3{,}0 \cdot 10^2$ und $K_2 = 0{,}50 \cdot 10^5$ an, BÖESEKEN und Mitarbeiter für Mannit $K_2 = 1{,}7 \cdot 10^{-4}$ und für Fruktose $K_2 = 1{,}1 \cdot 10^{-4}$. Ihre Konstanten sind mit $K_{HB}$ multipliziert.

Die Tabellen 1–4 zeigen einige typische Versuchsreihen. In den Figuren 2–5 sind die Messungen veranschaulicht.

Wenn undissoziierte $HBD_2$ in grösserer Menge gebildet wird, so würden [HB] und [D] kleiner und das linke Glied in 9) grösser als berechnet sein. Speziell bei hoher [D] ($>0{,}3$ C) und einer damit erhöhten $[H^+]$ wird sich eine Korrektion bemerkbar machen. Durch Zusatz von NaOH kann man die $[HBD_2]$ herabsetzen, und wenn man dann einen neuen Wert von 9) berechnet, so müsste er etwas oberhalb der Kurve liegen. Aus der Differenz liesse sich dann die undissoziierte $[HBD_2]$ berechnen. Die aus den Versuchen erhaltenen Werte liegen

### *Tabelle 1*

Mannit, kleine Konzentrationen. 20 ml $3{,}39 \cdot 10^{-2}$ C HB wird mit wachsender Menge von D versetzt, $a = 8{,}33 \cdot 10^{-6}$, E° = 356,9

| ml D | Emk mV | log [H+] | $[H^+] \cdot 10^5$ | $[HB] \cdot 10^2$ | $[D] \cdot 10^2$ | Das linke Glied in 9) |
|---|---|---|---|---|---|---|
| — | 32,8 | 5,477 | 0,334 | 3,39 | — | — |
| 0,705 | 60,6 | 5,008 | 0,982 | » | 0,355 | 10,1 |
| 1,800 | 79,0 | 4,698 | 2,005 | » | 0,868 | 15,6 |
| 3,69 | 94,7 | 4,432 | 3,70 | » | 1,666 | 25,2 |
| 11,58 | 117,4 | 4,049 | 8,93 | 3,38 | 4,10 | 54,5 |
| 14,69 | 121,4 | 3,981 | 10,44 | » | 4,79 | 63,0 |
| 19,30 | 125,4 | 3,914 | 12,19 | » | 5,65 | 72,3 |

### *Tabelle 2*

Fruktose, kleine Konzentrationen. 20 ml $1{,}695 \cdot 10^{-2}$ C HB wird mit wachsender Menge von D versetzt, $a = 5{,}64 \cdot 10^{-6}$, E° = 356,9

| ml D | Emk mV | log [H+] | $[H^+] \cdot 10^5$ | $[HB] \cdot 10^2$ | $[D] \cdot 10^2$ | Das linke Glied in 9) |
|---|---|---|---|---|---|---|
| — | 24,6 | 5,617 | 0,242 | 1,695 | — | — |
| 0,840 | 58,1 | 5,051 | 0,890 | 1,694 | 0,467 | 12,1 |
| 1,725 | 71,4 | 4,826 | 1,493 | 1,694 | 0,926 | 16,0 |
| 5,08 | 92,7 | 4,466 | 3,42 | 1,692 | 2,42 | 28,5 |
| 9,56 | 104,8 | 4,262 | 5,47 | 1,690 | 3,97 | 42,4 |
| 13,01 | 110,2 | 4,171 | 6,75 | 1,688 | 4,91 | 51,5 |
| 16,12 | 113,4 | 4,116 | 7,65 | 1,687 | 5,62 | 57,5 |
| 19,55 | 116,3 | 4,067 | 8,57 | 1,686 | 6,29 | 63,8 |

### *Tabelle 3*

Mannit, grosse Konzentrationen. 20 ml $1{,}565 \cdot 10^{-2}$ C HB wird mit wachsender Menge von D versetzt, $a = 6,\ 68 \cdot 10^{-6}$, E° = 357,1

| ml D | Emk mV | log [H+] | $[H^+] \cdot 10^5$ | $[HB] \cdot 10^2$ | $[D] \cdot 10^2$ | Das linke Glied in 9) |
|---|---|---|---|---|---|---|
| — | 20,8 | 5,686 | 0,206 | 1,565 | — | — |
| 1,125 | 109,3 | 4,190 | 6,45 | 1,559 | 4,45 | 57,2 |
| 2,41 | 128,1 | 3,872 | 13,43 | 1,552 | 8,96 | 118 |
| 4,155 | 140,3 | 3,666 | 21,6 | 1,543 | 14,34 | 189 |
| 8,22 | 153,9 | 3,436 | 36,6 | 1,528 | 24,3 | 320 |
| 16,93 | 165,3 | 3,244 | 57,0 | 1,508 | 38,2 | 496 |
| 25,78 | 170,5 | 3,156 | 69,8 | 1,495 | 47,0 | 609 |
| 32,51 | 173,0 | 3,113 | 77,1 | 1,488 | 51,6 | 679 |

*Tabelle 4*

Fruktose, grosse Konzentrationen. 20 ml $2{,}305 \cdot 10^{-2}$ C HB wird mit wachsender Menge von D versetzt, $a = 6{,}76 \cdot 10^{-6}$, E° = 359,9

| ml D | Emk mV | log [$H^+$] | [$H^+$] · $10^5$ | [HB] · $10^2$ | [D] · $10^2$ | Das linke Glied in 9) |
|---|---|---|---|---|---|---|
| — | 31,2 | 5,556 | 0,278 | 2,305 | — | — |
| 1,055 | 115,5 | 4,131 | 7,40 | 2,298 | 4,51 | 49,8 |
| 1,720 | 126,7 | 3,942 | 11,43 | 2,294 | 7,14 | 73,3 |
| 3,50 | 142,7 | 3,672 | 21,30 | 2,284 | 13,40 | 133 |
| 7,30 | 158,1 | 3,411 | 38,8 | 2,267 | 24,0 | 245 |
| 12,94 | 168,6 | 3,234 | 58,3 | 2,247 | 35,4 | 377 |
| 18,05 | 173,8 | 3,145 | 71,6 | 2,233 | 42,7 | 472 |
| 26,06 | 179,3 | 3,053 | 88,5 | 2,216 | 50,9 | 608 |

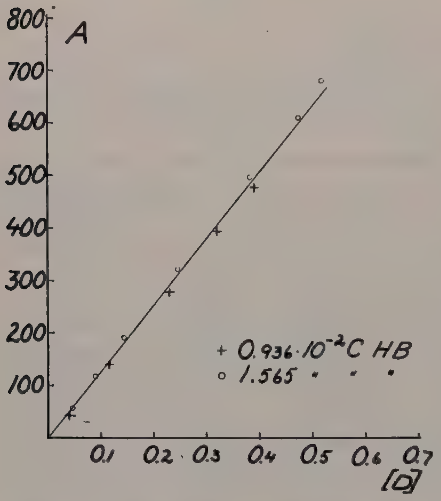


Fig. 4. Mannit, grosse Konzentrationen.

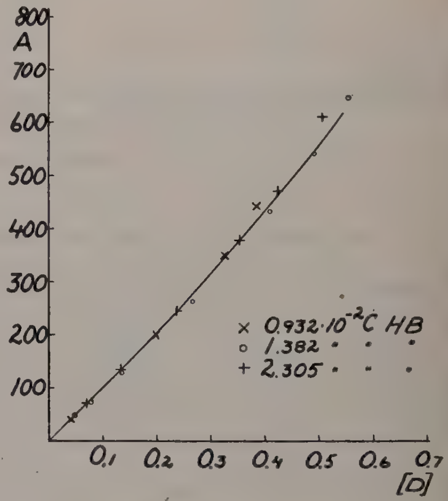


Fig. 5. Fruktose, grosse Konzentrationen.

indessen auf oder unterhalb der Kurve. Dies beweist, dass keine messbaren Mengen undissoziierter komplexer Säuren vorhanden sind. Auch der geradlinige Verlauf der Kurve ist eine Bestätigung dafür. Die gebildeten Komplexe sind starke Säuren und praktisch vollständig dissoziiert.

## Polarographische Messungen

Heyrovský und Smolér (8) zeigten, dass die Fruktose bei pH ~ 12 eine polarographische Stufe ergibt. Hierbei wird die Ketogruppe zu einer Hydroxylgruppe reduziert.

Wenn man annimmt, dass die Borsäure an das zweite Kohlenstoffatom der Fruktose gebunden ist, dann müsste es blockiert sein, und die komplexgebundene

Fruktose würde an dem Hg-Tropfen nicht reduziert werden können. Wir nehmen weiter an, dass die Senkung proportional der komplexgebundenen Fruktosemenge ist. (In der Besprechung wird diese Hypothese eingehender behandelt werden.) Daraus ergibt sich die Möglichkeit die Komplexkonstanten für die Bildung von $BD^-$ zu berechnen. Die totale Fruktose- und Borsäurekonzentration — sehr niedrig gehalten, um die Bildung von $BD_2^-$ zu vermindern — wird als bekannt vorausgesetzt.

Diese Methode ist natürlich nur mit einem reduzierbaren Polyalkohol anwendbar. Mannit, welches nur OH-Gruppen hat, wird nicht reduziert.

## Experimenteller Teil

Apparatur: Polarograph, von Radiometer, Kopenhagen.

Chemikalien: Fruktose und Borsäure, siehe oben; LiCl p. a. Baker; $LiOH \cdot 2\,H_2O$, Merck, U. S. A.

Die Lösungen enthielten 0,1 C LiCl und 0,01 C LiOH (pH ~ 12). Sie wurden 10 Minuten lang mit $N_2$ durchströmt. Die Versuche wurden bei Zimmertemperatur von 20° ± 1° ausgeführt.

## Besprechung

Bei pH ~ 12 liegt praktisch alle HB in Form von $B^-$ vor. Wir nahmen oben an, dass die Senkung der polarographischen Stufe proportional der komplexgebundenen Fruktosemenge ist. Wird die Gleichgewichtskonstante für die Bildung von $BD^-$ berechnet (Korrektion für $BD_2^-$ ist eingeführt), so erhält man als Mittelwert aus einer Anzahl Versuchsreihen $K_1 = 7{,}0 \cdot 10^2$. (Tabelle 5, und Fig. 6.). Die Streuung der Werte ist relativ gross, was auf die Schwierigkeit, die Stufenhöhe genauer als auf ± 1 mm abzulesen, zurückzuführen ist. Es zeigt sich eine gute Übereinstimmung zwischen dem polarographisch erhaltenen Mittelwert und dem Resultat, welches sich aus den Emk-Messungen (25° C) ergibt $= 6{,}9 \cdot 10^2$. Allerdings wurde in den Versuchen mit den beiden Methoden nicht genau dieselbe Temperatur eingehalten.

*Tabelle 5*

Die Mittelwerte vier verschiedener Versuchsserien $7{,}0 \cdot 10^2$ und $13{,}5 \cdot 10^2$.

| $D_{tot} \cdot 10^3$ | $B^-_{tot} \cdot 10^3$ | pH | Stufenhöhe | Differenz der Stufenhöhen | $BD^- + 2\,BD_2^- \cdot 10^3$ | $K_1 \cdot 10^{-2}$ ($B^-$ wird an $D^-$ gebunden) | $K_1 \cdot 10^{-2}$ ($B^-$ wird nicht an $D^-$ gebunden) |
|---|---|---|---|---|---|---|---|
| 1,056 | — | 11,91 | 59,9 | — | — | — | — |
| » | 0,1293 | 11,90 | 56,4 | 3,5 | 0,062 | 6,9 | 14,4 |
| » | 0,259 | 11,90 | 53,7 | 6,2 | 0,109 | 5,6 | 11,9 |
| » | 0,388 | 11,90 | 48,5 | 11,4 | 0,201 | 10,4 | 19,9 |
| » | 0,518 | 11,89 | 47,7 | 12,2 | 0,215 | 6,3 | 12,6 |
| » | 0,647 | 11,88 | 43,9 | 16,0 | 0,282 | 8,2 | 15,6 |
| » | 0,777 | 11,87 | 42,3 | 17,6 | 0,310 | 7,3 | 13,7 |
| » | 0,905 | 11,87 | 37,9 | 22,0 | 0,388 | 9,7 | 17,5 |
| » | 1,035 | 11,85 | 36,6 | 23,3 | 0,410 | 8,7 | 15,5 |
| » | 1,165 | 11,85 | 35,8 | 24,1 | 0,425 | 7,8 | 13,9 |
|  |  |  |  |  |  | Mittelwert 7,9 | Mw 15,0 |

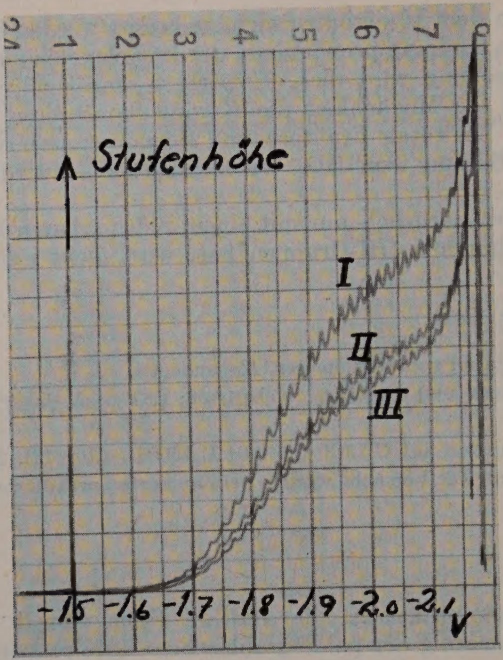


Fig. 6. I Die reine Fruktoselösung ($1{,}018 \cdot 10^{-3}$ C). II Die Fruktoselösung + $0{,}737 \cdot 10^{-3}$ C HB. III Die Fruktoselösung + $0{,}860 \cdot 10^{-3}$ C HB.

Hierbei wurde aber nicht in Erwägung gezogen, dass die Fruktose auch als eine schwache Säure angesehen werden muss, $D \rightleftharpoons D^- + H^+$. Michaelis (9) gibt $K_s = 8{,}8 \cdot 10^{-13}$ an. Dabei taucht wiederum die Frage auf, ob $B^-$ auch an $D^-$ gebunden werden könnte. Die Fruktose liegt in zwei Formen vor, in einer zyklischen und in einer offenen Form. In der offenen Form kommt eine Ketogruppe vor, die in der zyklischen Form in eine OH-Gruppe umgewandelt worden ist. Man dürfte annehmen können, dass das H-Atom dieser OH-Gruppe leichter beweglich als die anderen Wasserstoffatome ist. Bei Alkalizusatz wird wahrscheinlich dieses H-Atom abgespalten. Folglich könnte eine Ionisation die Ringstruktur stabilisieren. Da sich nach Zusatz von HB eine Senkung der polarographischen Stufe zeigt, so kann dies als ein Beweis dafür gelten, dass HB an das zweite Kohlenstoffatom gebunden wird.

Hermans (10) ist der Ansicht, dass die Komplexbildung unter Wasseraustritt und Ringschluss vor sich geht. Dieses ist nun nicht mehr möglich, da das Fruktosemolekül am zweiten Kohlenstoffatom ionisiert ist. Berechnet man die Komplexkonstante unter der Voraussetzung, dass $B^-$ nur an undissoziierte Fruktose angelagert wird, so erhält man den Wert $13{,}5 \cdot 10^2$. Dieser ist doppelt so gross wie der aus den Emk-Messungen berechnete Wert. Dies deutet darauf hin, dass eine Komplexbildung sowohl mit ionisierter als auch mit nicht ionisierter Fruktose eingetreten ist, was durchaus möglich ist, wenn die Komplexbildung durch *Wasserstoffbrücken* zustandekommt.

Um das polarographische Verhalten des $D^-$-Ions zu untersuchen, wurde die Stufenhöhe bei verschiedenen $[H^+]$ gemessen. In der Fig. 7 ist auf der Y-Achse

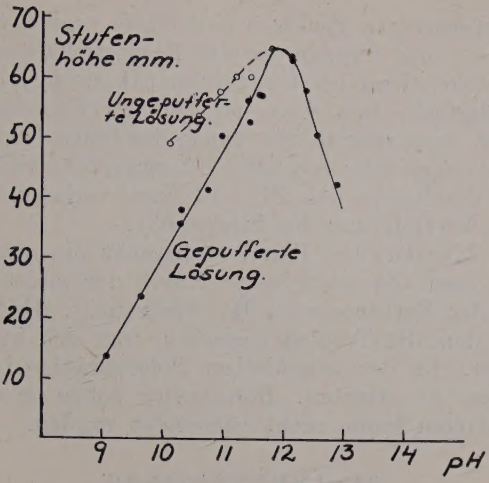
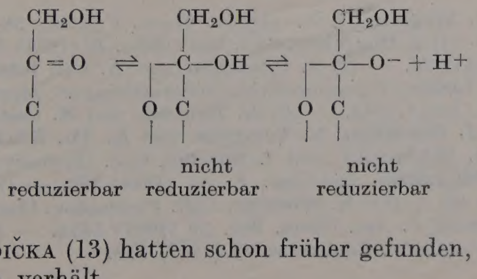


Fig. 7. Abhängigkeit der Stufenhöhe von pH.

die Stufenhöhe bei konstanter [D] eingezeichnet worden und auf der X-Achse das pH. Bis zum pH = 12 sind Karbonat- oder Phosphatpuffer verwandt worden. Oberhalb pH = 12 puffert LiOH selbst.

In den meisten Fällen wird die Stufenhöhe durch die Diffusionsgeschwindigkeit des reduzierbaren Stoffes bestimmt. Bei der Fruktose liegen aber die Verhältnisse anders. Wiesner (11) zeigte, dass die polarographische Stufenhöhe durch die Geschwindigkeit, mit der die Umlagerung von der zyklischen Form in die reduzierbare (offene?) Form vor sich geht, bestimmt wird.

Die pH-Abhängigkeit der Stufenhöhe weist ein Maximum auf (Fig. 7). Man kann die Form der Kurve folgendermassen erklären. $OH^-$ katalysiert die Umlagerung. In ähnlicher Weise beeinflusst $OH^-$ auch die Mutarotation der Glukose (12). Wenn das pH steigt, nimmt also auch die Stufenhöhe zu; jedoch wird durch die Dissoziation der Fruktose als Säure die freie Fruktosemenge vermindert, schliesslich — bei hohen pH-Werten — so weitgehend, dass die Stufenhöhe abnimmt. Man muss dabei annehmen, dass das Ion nicht reduziert wird. Die Senkung tritt bei pH = 12 in Erscheinung, was mit der Dissoziationskonstante der Fruktose, $K_s = 8{,}8 \cdot 10^{-13}$ übereinstimmt. Es kann also nur das undissoziierte Fruktosemolekül mit offener Kette reduziert werden.



$$\begin{array}{ccccc} CH_2OH & & CH_2OH & & CH_2OH \\ | & & | & & | \\ C=O & \rightleftharpoons & |\!-\!C\!-\!OH & \rightleftharpoons & |\!-\!C\!-\!O^- + H^+ \\ | & & |\quad | & & |\quad | \\ C & & O\ \ C & & O\ \ C \\ | & & |\quad | & & |\quad | \\ \text{reduzierbar} & & \text{nicht reduzierbar} & & \text{nicht reduzierbar} \end{array}$$

Veselý und Brdička (13) hatten schon früher gefunden, dass sich der Formaldehyd ganz ähnlich verhält.

Dass nur die undissoziierte Fruktose eine Stufe ergibt, ändert nichts daran, dass nach HEYROVSKÝ und SMOLÉR (9) eine Proportionalität zwischen $[D_{tot}]$ und der Stufenhöhe besteht, denn bei konstanten pH wird [D] proportional $[D_{tot}]$. Ebenso ist es verständlich, dass wenn ein Teil der Fruktose von $B^-$ gebunden wird, die Stufenhöhe proportional der Menge der freien Fruktose ist. Bei Zusatz von HB zu einer Fruktoselösung von pH = 12 verändert sich das pH unbedeutend, $< 0{,}1$ pH, aber da die Kurve bei pH = 12 flach verläuft, bedeutet eine kleine pH-Änderung keine Veränderung der Stufenhöhe.

Von den beiden Kurven der Fig. 7 entspricht die ausgezogene Kurve der gepufferten Lösung und die gestrichelte Kurve der nicht gepufferten Lösung. Bei der Reduktion der Fruktose wird $H^+$ verbraucht. Dadurch wird die ungepufferte Lösung an dem Hg-Tropfen basischer, was sich in einer Zunahme der Stufenhöhe offenbart. In der organischen Polarographie ist es notwendig mit gepufferten Lösungen zu arbeiten. Boratpuffer sollen in den Fällen, wo eine Komplexbildung eintreten kann, nicht verwendet werden.

## ZUSAMMENFASSUNG

Die Komplexkonstanten für die Bildung von $BD^-$ und $BD_2^-$ sind für Mannit und Fruktose berechnet. Die Emk-Messungen gaben die folgenden Werte (bei 25° C):

Mannit: $K_1 = 5{,}6 \cdot 10^2$; $K_2 = 1{,}16 \cdot 10^5$
Fruktose: $K_1 = 6{,}9 \cdot 10^2$; $K_2 = 0{,}83 \cdot 10^5$

Das Resultat der polarographischen Messungen (bei 20° C) war:

Fruktose: $K_1 = 7{,}0 \cdot 10^2$

Die komplexen Säuren HBD und $HBD_2$ sind starke Säuren und praktisch vollständig dissoziiert. Die Gegenwart von $B_2D^-$ oder $B_2D_2^-$ und wahrscheinlich auch von $BD_3^-$ bei Fruktose ist nachgewiesen worden.

Bei Gegenwart von Borat sinkt die polarographische Stufe der Fruktose proportional der komplexgebundenen Fruktosemenge. Die Borsäure wird an das zweite Kohlenstoffatom der Fruktose gebunden. Die Möglichkeit einer Bindung des Borations an ein negatives Fruktoseion ist besprochen worden. Die pH-Abhängigkeit der polarographischen Stufenhöhe der Fruktose ist untersucht und erklärt worden.

Herrn Professor Dr K. MYRBÄCK sage ich für seine wertvollen Anregungen und sein liebenswürdiges Interesse für diese Arbeit meinen ergebensten Dank. Herrn Dozent Dr. C. BROSSET und Herrn Dr. B. AURIVILLIUS bin ich für Diskussionen über die experimentelle Metodik grossen Dank schuldig.

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LITERATUR: (1) **G. Magnanini,** Z. physik. Chem. *6* (1890) 58; ebenda *9* (1892) 230; ebenda *11* (1893) 281. — (2) **J. Böeseken,** Rec. trav. chim. *40* (1921) 553. — (3) **H. Schäfer,** Z. anorg. Chem. *247* (1947) 96. — (4) **J. Böeseken** und **A. van Rossem,** Rec. trav. chim. *30* (1911) 392. — (5) **I. Leden,** Potentiometrisk undersökning av några kadmiumsalters komplexitet, Akad. avhandl., Lund 1943. — (6) **A. Deutsch** und **S. Osolin,** J. Am. Chem. Soc. *71* (1949) 1637. — (7) **J. Böeseken, N. Vermaas** und **A. Th. Küchlin,** Rec. Trav. chim. *49* (1930) 711. — (8) **J. Heyrovský** und **I. Smolér,** Coll. Czechoslov. Chem. Communs. *4* (1932) 521. — (9) **L. Michaelis,** Biochem. Z. *65* (1914) 360. — (10) **P. H. Hermans,** Z. anorg. Chem. *142* (1925) 83. — (11) **K. Wiesner,** Coll. Czechoslov. Chem. Communs. *12* (1947) 64. — (12) **C. S. Hudson,** J. Am. Chem. Soc. 29 (1907) 1572. — (13) **K. Veselý** und **R. Brdička,** Coll. Czechoslov. Chem. Communs. *12* (1947) 313.

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